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Biological Effects of the Grassland Bypass Project

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Abstract

Biological monitoring continued for the Grassland Bypass Project's sixth year of operation at seven sampling sites (Figure 1). Results presented below cover a 15-month period from October 2001 through December 2002. All whole body composite samples (small fish, invertebrates, and vegetation) results are presented as average selenium concentrations (mg/kg) based on dry tissue weight. All muscle tissue composite samples (mainly carp) results are presented as average selenium concentrations (mg/kg) based on wet tissue weight.

Selenium concentrations in whole-body fish and invertebrates sampled in Mud Slough below the outfall of the San Luis Drain (SLD) frequently exceeded thresholds of concern as presented in Table 1. However, for the 15-month period covered in this report, average selenium concentrations of all composite fish samples from Mud Slough sites either decreased significantly (Site D), increased significantly (Site E), or did not change (Site I2) compared to Water Year (WY) 2001.

The first site in Mud Slough contaminated with drainage water from the Grassland Drainage Area is Site D. The concentration of selenium in 18 of 20 composite samples of small fish caught at this site during the fifteen month study period exceeded the 4 mg/kg (dry weight) threshold of concern (Figure 10). The concentration of selenium in inland silversides caught March 2002 and in fathead minnows caught August 2002 exceeded the 9 mg/kg (dry weight) threshold of toxicity (Figure 10). The concentration of selenium in bullfrogs caught in August 2002 was below the 3 mg/kg threshold of concern (Figure 12). The concentration of selenium in red crayfish caught November 2001 exceeded the 3 mg/kg threshold of concern (Figure 13). The overall hazard of selenium to the ecosystem (Lemly's index) continued to be high in the reach of Mud Slough below the SLD outfall (Table 4)

At a backwater site further downstream from the outfall (Site I2), the average selenium concentrations in all 20 composite samples of small fish caught during the fifteen month study period exceeded the 4 mg/kg concern threshold (Figure 14). All four composite samples caught during August 2002 exceeded the 9 mg/kg toxicity threshold (Figure 14). The average concentration of selenium in carp caught in June and August 2002 was slightly below to the 9 mg/kg toxicity threshold; seven samples of Sacramento blackfish collected November 2001 and November 2002 were below the 3 mg/kg threshold of concern (Figure 15). The concentration of selenium in waterboatmen invertebrates caught at this site was above the 3 mg/kg threshold of concern during three of four sampling events (Figure 16). The concentration of selenium in red crayfish caught November 2001 was above the 7 mg/kg threshold of toxicity; the concentration of selenium in red crayfish caught one year later was lower, but above the 3 mg/kg threshold of concern (Figure 16).

At a site further downstream in Mud Slough just above its confluence with the San Joaquin River, (Site E), selenium in whole-body fish exceeded the 9 mg/kg threshold of toxicity on four of five sampling events during the fifteen month study period (Figure 17). The concentration of selenium in red crayfish exceeded the threshold of toxicity in samples collected in August and December 2002 (Figure 18). The selenium concentrations in carp muscle tissue collected at this site during November 2001 and August 2002 exceeded the 2 mg/kg (wet weight) human health consumption guideline (Figure 27).

At a sampling site on Mud Slough above the outfall (Site C), the selenium concentration of nine of seventeen samples of small fish collected at this site were above the 4 mg/kg concern threshold (Figure 6). The concentration of selenium in medium-sized fish, bullfrogs, and invertebrates remained within the no-effect level (Figures 7, 8, and 9).

In Salt Slough, where drainwater has been removed by the GBP, average selenium concentrations in small and medium fish and invertebrates remained at no-effect levels during the fifteen month study period (Figures 2, 3 and 5). The concentration of selenium in bullfrogs caught August 2002 exceeded the 4 mg/kg threshold of concern (Figure 4). The overall hazard of selenium to the ecosystem (Lemly's index) was low in Salt Slough (Table 4).

In the San Joaquin River upstream (Site G) of the Mud Slough discharge, selenium concentrations in whole-body fish remained below the concern threshold of 4 mg/kg (dry weight) (Figure 19). Selenium concentrations in all invertebrates collected from this site remained below the 3 mg/kg (dry weight) threshold of concern for invertebrates as prey items (Figure 20). The selenium concentration in all carp muscle tissues collected at this site during the fifteen month study period were below the 2 mg/kg (wet weight) human health consumption guideline (Figure 28).

However, in the San Joaquin River downstream of the Mud Slough discharge (Site H), selenium concentrations in whole-body fish exceeded the concern threshold of 4 mg/kg (dry weight) in samples collected in March and December 2002 (Figure 21). Selenium concentrations in red crayfish collected from this site exceeded the 3 mg/kg (dry weight) concern threshold in samples collected in November 2001 and December 2002 (Figure 22). The concentration of selenium in all samples of carp muscle tissue collected at this site during the fifteen month study period was below the 2 mg/kg (wet weight) human health consumption guideline (Figure 29).

The selenium concentrations in all bird eggs collected during the fifteen month study period in the Salt Slough area and the Mud Slough area were within the no effect range (Figure 31).

Selenium concentrations in seeds collected at sites C, F and I2 in August 2002 were below the analytical reporting limit of 0.2 mg/kg (dry weight). The concentration of selenium in swamp timothy seed heads collected at Site D in August 2002 was above the 3 mg/kg threshold of concern as diet for birds. All seed samples collected at sites E, G and H were within the dietary no-effect level as diet for birds (Figure 30).

The boron concentration in one composite seed sample from the bank of Salt Slough was just slightly above the threshold of concern. The boron concentration in one of three plant samples collected from Mud Slough sites below the SLD outfall was above the 30 mg/kg (dry weight) threshold of concern. Both composite seed samples collected along Mud Slough above the outfall (Site C) were above the boron threshold of concern. The boron concentration in all samples collected from the San Joaquin River near Fremont Ford (Site G) was below the 30 mg/kg (dry weight) threshold of concern. The concentration of boron in seeds collected at the San Joaquin River at Hills Ferry (Site H) was above the 30 mg/kg (dry weight) threshold of concern.

Introduction

Project History

In 1985 the SLD was closed due to deaths and developmental abnormalities of waterbirds at a reservoir in the Kesterson National Wildlife Refuge at the terminus of the SLD. The SLD, constructed by the U.S. Bureau of Reclamation (USBR), had been conceived as a means to dispose of agricultural drainwater generated from irrigation with water supplied by the federal Central Valley Water Project. However, due to environmental concerns and budget constraints, the SLD had never been completed as originally planned. The constructed portion of the SLD had been used only to convey subsurface agricultural drainwater from the Westlands Water District in the western San Joaquin Valley. Farms in the adjacent Grassland Drainage Area (GDA) never used the SLD, but discharged subsurface drainwater through wetland channels in the Grassland Water District, San Luis National Wildlife Refuge Complex, and the China Island Unit of the North Grasslands Wildlife Area (Refuges) to the San Joaquin River. This drainwater contains elevated concentrations of selenium, boron, chromium, and molybdenum, and high concentrations of various salts (CEPA, 2000) that disrupt the normal ionic balance of affected aquatic ecosystems (SJVDP, 1990b).

Discharge from GDA farms was unaffected by the closure of the SLD, and drainage continued to contaminate Refuge water delivery channels after the closure of the SLD and Kesterson Reservoir in 1986. To address this problem, a proposal to use a portion of the SLD and extend it to Mud Slough, a natural waterway in the Refuges, was implemented by the USBR in September 1996 with support from other federal and state agencies (USBR, 1995; USBR and SLDMWA 1995; USBR et al., 1995). This project, known as the Grassland Bypass Project (GBP), diverts agricultural drainwater from GDA farms into the lower 28 miles of the SLD and thence into the lower portion of Mud Slough (about six miles). The GBP has removed drainwater from more than 90 miles of wetland water supply channels, including Salt Slough, and allows the Refuges full use of water rights to create and restore wetlands on the Refuges. The GBP, as currently implemented, continues to affect the northernmost six miles of Mud Slough and the reach of the San Joaquin River between Mud Slough and the Merced River. However, as phased-in load reduction goals are achieved by GDA farmers, these effects are expected to be reduced. An essential component of the GBP is a monitoring program that tracks contaminant levels and effects in water, sediment, and biota to ensure that the overall effect of the GBP is not a net deterioration of the ecosystems in the area affected by the GBP.

Contaminants of Concern

In the aftermath of the deaths and developmental abnormalities of birds at Kesterson Reservoir in the early 1980s, studies definitively traced the cause to selenium in the agricultural subsurface drainwater in the reservoir (Suter, 1993). Because of this, and because of the well-known history of death, teratogenesis, and reproductive impairment caused by selenium in agricultural drainwater elsewhere (reviewed in Skorupa, 1998), the primary contaminant of concern in this monitoring program is selenium. Other inorganic constituents of potential toxicological interest in drainage water include boron, molybdenum, arsenic and chromium (Klasing and Pilch, 1988; SJVDP, 1990a; CVRWQCB, 1998).

Selenium Ecological Risk Guidelines

The assessment of the risks that selenium poses to fish and wildlife can be difficult due to the complex nature of selenium cycling in aquatic ecosystems (Lemly and Smith, 1987). Early assessments developed avian risk thresholds through evaluating bird egg concentrations and relating those to levels of teratogenesis (developmental abnormalities) and reproductive impairment (Skorupa and Ohlendorf, 1991). In 1993, to evaluate the risks of the Grassland Bypass Project on biotic resources in Mud and Salt Sloughs, a set of Ecological Risk Guidelines based on selenium in water, sediment, and residues in several biotic tissues were developed by a subcommittee of the San Luis Drain Re-Use Technical Advisory Committee (CAST, 1994; Engberg, et.al., 1998). These guidelines (as recently modified: Table 1) are based on a large number of laboratory and field studies, most of which are summarized in Skorupa et al. (1996) and Lemly (1993). In areas where the potential for selenium exposure to fish and wildlife resources exists, these selenium risk guidelines can be used to trigger appropriate actions by resource managers, regulatory agencies, and dischargers. For the GBP the selenium risk guidelines have been divided into three threshold levels: No Effect, Concern, and Toxicity.

In the No Effect range risks to sensitive species are not likely. As new information becomes available it should be evaluated to determine if the No Effect level should be adjusted. Since the potential for selenium exposure exists, periodic monitoring of water and biota is appropriate.

Within the Concern range there may be risk to species sensitive to elevated contaminant concentrations in water, sediment, and biota, and should be monitored on a regular basis. Immediate actions to prevent selenium concentrations from increasing should be evaluated and implemented if appropriate. Long-term actions to reduce selenium risks should be developed and implemented. Research on effects on sensitive or listed species may be appropriate.

Within the Toxicity range, adverse affects are more likely across a broader range of species, and sensitive or listed species would be at greater risk. These conditions will warrant immediate action to reduce selenium exposure through disruption of pathways, reduction of selenium loads, or other appropriate actions. More detailed monitoring, studies on site-specific effects, and studies of pathways of selenium contamination may be appropriate and necessary. Long-term actions to reduce selenium risks should be developed and implemented.

The guidelines (except those for avian eggs) are intended to be population based. Therefore they should be used for evaluating population means rather than contaminant concentrations in individuals.

Warmwater Fish

The warmwater fish guidelines (Table 1) refer to concentrations of selenium in warmwater fish that adversely affect the fish themselves. The original 1993 fish guidelines have been replaced by explicitly "warmwater fish" guidelines in recognition of the evidence from the literature that coldwater fish (salmon and trout) are more sensitive to selenium than warmwater fish and that GBP monitoring data available is limited to warmwater fish. Although a coldwater fish guideline is not proposed here, a discussion of selenium effects on coldwater fish is provided in this section since the best information currently available happens to be very site-specific to the GBP area (Merced River and downstream San Joaquin River).

The concern threshold for warmwater fish has been kept at 4 mg/kg (all fish data are whole body, dry weight). Experimental data reported in the literature may be interpreted to support a range of thresholds around this value. In particular, bluegill sunfish dietary and waterborne toxicity data in Cleveland et al. (1993) can be used to support warmwater fish concern thresholds of 3.3 mg/kg, 3.4 mg/kg, 3.9 mg/kg, or 5.9 mg/kg. Bluegill sunfish are warmwater fish that are found in the sloughs in the GBP area, and the Cleveland et al. (1993) study yielded the best available data on warmwater fish toxicity applicable to GBP.

Cleveland et al. (1993) found no adverse effects after 59 days of exposure to concentrations of dietary selenium that resulted in a bluegill tissue concentration of 2.7 mg/kg (NOEC). Fifty nine days of exposure to dietary concentrations that resulted in tissue concentrations of 4.2 mg/kg (LOEC) caused a significant increase in mortality relative to controls. Following the USEPA method (Stephan et al., 1985) employed by DeForest et al. (1999), the tissue threshold is calculated as the geometric mean of the NOEC and the LOEC. Application of the USEPA procedure to these data yields a toxicity threshold of 3.4 mg/kg. A similar analysis of a water-borne selenium exposure experiment (Cleveland et al., 1993) yields a threshold value of 3.3 mg/kg.

Other data in Cleveland et al. (1993) may be interpreted to support a threshold closer to 4 mg/kg or a threshold of 5.9 mg/kg. The experiments of Cleveland et al. (1993) suggest that selenium concentrations in fish tissues do not reach equilibrium until at least 90 days of dietary exposure (Figure 3 in Cleveland et al., 1993). This appears consistent with the finding, summarized below, that in the field, selenium concentrations in fish are best predicted by water concentrations averaged over the entire period of one to seven months prior to the date the fish is sampled. In deriving a tissue threshold, there then appears to be some support for using the relationship between dietary concentration and tissue concentration at 90 days rather than 59 days. After 90 days of dietary exposure bluegill with a tissue selenium concentration of 3.3 mg/kg did not exhibit adverse effects that were significantly greater than controls, but bluegill with a tissue concentration of 4.6 mg/kg experienced significantly increased mortality. Bluegill with a tissue concentration of 7.5 mg/kg had three times the mortality of controls, but that difference in mortality was not statistically significant at the 95% level of confidence (Table 4 and Figure 3 in Cleveland et al., 1993). However, the condition factor (a measure of weight relative to length) of the fish at 7.5 mg/kg, was significantly worse than controls. Depending on whether or not the significant mortality at a tissue concentration of 4.7 mg/kg is treated as anomalous, the LOEC would be either 4.7 mg/kg or 7.5 mg/kg. Corresponding thresholds would be 3.9 mg/kg (geometric mean of 3.3 mg/kg and 4.6 mg/kg) or 5.9 mg/kg (geometric mean of 4.6 mg/kg and 7.5 mg/kg) respectively. Given the range of possible threshold values discussed above, the concern threshold of 4 mg/kg listed in Table 1 was not changed from the original 1993 threshold. However, considering that these data do not include adverse effects on reproduction which that may occur at lower concentrations, this threshold may not be fully protective of sensitive warmwater fish species.

The toxicity threshold for warmwater fish (whole body) of 9 mg/kg is recommended by DeForest et al. (1999). In the analysis of DeForest et al. (1999) the threshold represents an EC10, that is, the concentration at which 10 percent of fish are affected. DeForest et al. (1999) excluded some toxicity data from their analysis that could support a lower threshold (Cleveland et al., 1993). Also, reproductive impairment may occur at lower selenium concentrations, but too few data are available to do a similar analysis on this effect. Therefore, this Toxicity threshold may not be fully protective of sensitive warmwater fish species.

Coldwater Fish

Testing fall run chinook salmon from the Merced River, Hamilton et al. (1990) found that salmon fry growth was significantly reduced compared to controls after 30 and 60 days of being fed a diet (containing mosquitofish from the SLD) having a selenium concentration of 3.2 mg/kg dry weight. After 90 days of that diet, the selenium concentration in the salmon fry averaged 2.7 mg/kg whole body, dry weight. This fish tissue concentration was the lowest observable effect concentration (LOEC) The no observable effect concentration (NOEC) in salmon fry tissue was 0.8 mg/kg. Following the USEPA method (Stephan et al., 1985) employed by DeForest et al. (1999), the tissue threshold is calculated as the geometric mean of the NOEC and the LOEC. This procedure applied to the Hamilton et al. (1990) SLD data yields a threshold of 1.5 mg/kg (geometric mean of 0.8 and 2.7 mg/kg). It should be noted that this threshold may incorporate the interacting effects of other toxic constituents of drainwater that may have been assimilated by the SLD mosquitofish that were used as feed in the Hamilton, et al.(1990) experiments. Furthermore, at the time of these experiments (1985), the SLD held agricultural drainwater from the Westlands, an area adjacent to the Grasslands area. Therefore, although these are the most site-specific selenium toxicity data available, these data may not perfectly match the current risk of toxicity to coldwater fish in the San Joaquin River due to agricultural drainwater from the GBP. Although the sloughs affected by the GBP have coldwater beneficial uses designated by the Central Valley Regional Water Quality Control Board, the fish community principally consists of warmwater species. A temporary barrier is installed seasonally across the San Joaquin River to exclude chinook salmon (a coldwater species) from these sloughs and from the San Joaquin River upstream of its confluence with the Merced River. Additionally, any application of the coldwater fish risk guidelines should take into account the fact that many coldwater fish are anadromous, and therefore feed in the selenium-contaminated portion of the San Joaquin River for a limited period of time-- a brief period in their juvenile stage as they migrate downstream to the ocean.

A toxicity threshold for coldwater fish (whole body) of 9 mg/kg has been recommended by DeForest et al. (1999). In their analysis, the toxicity threshold represents an EC10, that is, the concentration at which 10 percent of fish are affected. DeForest et al. (1999) excluded site-specific and longer term data (Hamilton et al., 1990) which could support lower thresholds. For example, to derive their toxicity threshold for coldwater fish, DeForest et al. (1999) used only the 60 day growth data in Hamilton et al. (1999); they disregarded the 90 day mortality data in Hamilton et al. (1999) that would have yielded a toxicity threshold (corresponding to10% mortality) of 1.7 mg/kg. In addition, the DeForest et al. (1999) analysis focused on growth and mortality. Reproductive impairment may occur at lower selenium concentrations, but too few data are available to do a similar analysis on this effect. Therefore, this threshold may not fully protect sensitive coldwater fish species.

Vegetation and Invertebrates

The guidelines for vegetation (as diet) and invertebrates (as diet) refer to selenium concentrations in plants and invertebrates affecting birds that eat these items. These guidelines are mainly based on experiments in which seleniferous grain or artificial diets spiked with selenomethionine were fed to chickens, quail or ducks resulting in reproductive impairment

(Wilber, 1980; Martin, 1988; Heinz, 1996). The Concern threshold for vegetation is 3 mg/kg (dry weight) and the Toxicity threshold is 7 mg/kg. The invertebrate concern threshold and toxicity threshold are the same as those for vegetation.

Water

Fish and wildlife are much more sensitive to selenium through dietary exposure from the aquatic food chain than by direct waterborne exposure. Therefore the guidelines for water reflect water concentrations associated with threshold levels of food chain exposure (Hermanutz et al., 1990; Maier and Knight, 1994), rather than concentrations of selenium in water that directly affect fish and wildlife. The concern threshold is 2 μg/L and the toxicity threshold is 5 μg/L.

Sediment

As with water, the principal risk of sediment to fish and wildlife is via the aquatic food chain. Therefore the sediment guidelines are based on sediment concentrations as predictors of adverse biological effects through the food chain (USFWS, 1990; Van Derveer and Canton, 1997). The concern threshold for sediment (dry weight) is 2 mg/kg and the toxicity threshold is 4 mg/kg.

Bird Eggs

Bird eggs are particularly good indicators of selenium contamination in local ecosystems (Heinz, 1996). However, the interpretation of selenium concentrations in bird eggs in the GBP area is complicated by the proximity of contaminated and uncontaminated sites and by the variation in foraging ranges among bird species. Relative to the guidelines originally used for the GBP, the guidelines used here for individual bird eggs have been revised upward based on recent studies of hatchability of ibis, mallard, and stilt eggs (Henny and Herron, 1989; Heinz, 1996; USDI-BOR/FWS/GS/BIA, 1998). The concern threshold has been raised from 3 to 6 mg/kg dry weight, and the toxicity threshold has been raised from 8 to 10 mg/kg dry weight.

Selenium Ecological Risk Index

Several years after the risk guidelines were developed for the GBP, Lemly (1995, 1996) published a risk index designed to provide an estimate of ecosystem-level effects of selenium. Lemly's assessment procedure sums the effects of selenium on various ecosystem components to yield a characterization of overall hazard to aquatic life. The procedure involves determining an index of toxicity for each component, then adding these indexes together to yield a single index, often known as the Lemly Index. In contrast to the ecological risk guidelines outlined in Table 1, the component indexes of the Lemly Index are based on maximum contaminant concentrations rather than means. Therefore, the Lemly Index is sensitive to brief spikes in contaminant levels, but is unaffected by prevailing contaminant levels. Furthermore, the Lemly Index is strongly dependent on sampling periods and sampling frequency, yet Lemly provided no sampling protocol. For these reasons, there is a need to develop a new protocol and index that replaces Lemly's categorical rating format (low, medium, high) with a direct estimate of the probability of adverse effects (e.g.10%+ probability of reproductive impairment). Despite the weaknesses of the Lemly Index, we continue to use it for comparative purposes as long as it remains the best available overall index of the ecological risk of selenium.

Boron Ecological Risk Guidelines

The dietary and tissue concentrations of boron associated with toxic effects on fish and wildlife are not as well known as for selenium. The effects of dietary exposures and waterborne exposures (without dietary exposures) are known for some taxa (Table 2), but there are as yet no definitive data associating tissue concentrations with adverse effects in fish and invertebrates. Boron concentrations as low as 0.1 mg/l in water may adversely affect reproduction of sensitive fish species (review in NIWQP, 1998).

Methods

The role of the California Department of Fish and Game (CDFG) and the United States Fish and Wildlife Service (USFWS) in this interagency program is to implement the bio-monitoring portion of the Compliance Monitoring Program. The methods used by the CDFG and USFWS are described in the Quality Assurance Project Plan for Use and Operation of the Grassland Bypass Project (QAPP; Entrix, Inc., 1997). These methods are also based on standard operating procedures described in Standard Operation Procedures for Environmental Contaminant Operations (USFWS, 1995) and standards used by the other agencies participating in the compliance monitoring program. Deviations from the QAPP that have occurred since 1996 will be discussed later in this section.

To obtain baseline data for this Project, the USFWS began sampling in March 1992, after the reuse of the SLD was initially proposed by the USBR in 1991. The CDFG began sampling in August of 1993. USFWS and CDFG sampling plans before the reopening of the SLD and the early drafts of the monitoring plan were mutually influencing. Therefore, methods used by both agencies before the final approval of the QAPP are, except for a few minor differences, identical to the methods ultimately approved by the Data Collection and Reporting Team. The sampling schedule, though, as discussed below, now follows a regular timetable.

Due to the 2001 Waste Discharge Requirement Monitoring and Reporting Order, this report covers a fifteen month study period between October 2001 and December 2002.

Matrices Sampled

Samples of the biota were collected at each site and analyzed for selenium and boron. Aquatic specimens were collected with hand nets, seine nets and by electro fishing. Mosquitofish (Gambusia affinis), inland silversides (Menidia beryllina), red shiners (Cyprinella lutrensis), fathead minnows (Pimephales promelas), carp (Cyprinus carpio), white catfish (Ameiurus catus), and green sunfish (Lepomis cyanellus) were the principal species of fish collected. Waterboatmen (family: Corixidae), backswimmers (family: Notonectidae), and red crayfish (Procambarus clarkii) were the principal invertebrates collected. Separation of biological samples from unwanted material also collected in the nets was accomplished by using stainless steel or Teflon sieves, and glass (or enamel) pans pre-rinsed with de-ionized water then native water. To the extent possible, three replicate, composite samples (minimum 5 individuals totaling at least 2 grams for each composite) of each primary species listed above were collected, but other species were also collected. Fish species were analyzed as composite whole-body samples except as noted below. Estimates of a conversion factor for relating selenium concentration in skeletal muscle (M) to whole-body concentrations (WB) range from M=0.6xWB for many freshwater fish (Lemly and Smith, 1987) to M=0.045+1.23xWB for bluegills and M=-0.39+1.32xWB for largemouth bass (Saiki et al., 1991).

Between 1992 and 1999, frog tadpoles occasionally collected from Mud Slough and Salt Slough sites were archived. In 1999 these archived samples were analyzed. Additional samples were collected and analyzed from these sites in 2000 and 2001.

Analyses of fish samples collected from the San Joaquin River (Sites G and H) and Mud Slough (Sites C, D, I2 and E) were prioritized to first meet the objectives of the Compliance Monitoring Plan (Section 4.5.1.4). Supplemental fish samples were analyzed only when baseline biota target species and sample sizes could not be obtained.

In WY 1999, 2000, and 2001 several samples of fish and invertebrates submitted for analysis were of insufficient mass to permit individual measurement of the water content (percent moisture) of the sample, a measurement used to calculate the dry weight selenium concentration in the sample. For these samples (designated with asterisk on the graphs), an average percent moisture was calculated from the percent moisture measurements of comparable samples in the closest possible conditions of sampling location, time, species, and size of organism. This average percent moisture was used to calculate the dry weight selenium concentration. Selenium concentrations discussed in text and displayed in figures below are averages of composite sample concentrations except for bird eggs and except where otherwise stated.

The seed heads of wetland plants that provide food for waterfowl were collected along the sloughs in the late summer of the years 1995-2002. This plant material was archived for later analysis.

Waterfowl and/or shorebird eggs, depending on availability, were collected from areas adjacent to Mud Slough and the SLD in the spring of each year from 1996 through 2002. In addition, in 1992 snowy egret and black-crowned night heron eggs were collected at East Big Lake, which has served as a reference sampling site for the USFWS. Bird eggs were analyzed individually, and the results are discussed and displayed below as individual concentrations and geometric means.

Graphs of whole-body and avian egg selenium concentrations presented in this report include indications of the threshold concentrations delimiting the risk ranges listed above (Table 1). The threshold between the No Effect Zone and the Concern Zone is indicated by a horizontal line of short dashes; the Toxicity threshold is marked on each graph by a horizontal line of long dashes.

All biota samples were kept on ice or on dry ice while in the field then kept frozen to Zero degrees centigrade C during storage and shipment. For all samples, after freeze drying, homogenization, and nitric-perchloric digestion, total selenium was determined by hydride generation atomic absorption spectrophotometry and boron was determined by inductively coupled (argon) plasma spectroscopy.

Sampling Sites

Between 1992 and 1999 biological samples have been collected from two sites on Salt Slough, five sites on Mud Slough, two sites in the SLD, two sites on the San Joaquin River, and one reference site that does not receive selenium-contaminated drainwater (East Big Lake). Beginning in 1995, sampling efforts were concentrated on the seven sites (Figure 1) identified in the Compliance Monitoring Plan: four sites on Mud Slough (C, D, E, and I), one on Salt Slough (F) and two San Joaquin River sites (G and H). Site C is located upstream of where the Grassland Bypass discharges into Mud Slough. Site D is located immediately downstream of the discharge point. Site I is a small, seasonally flooded backwater area fed by Mud Slough and is located approximately 1 mile downstream from Site D. Site E is located further downstream where Mud Slough crosses State Highway 140. To assess the mitigative effects of drainwater removal from Salt Slough, one sample point, Site F, is located on the San Luis National Wildlife Refuge approximately 2 miles upstream of where State Highway 165 crosses Salt Slough. Site G is located on the San Joaquin River at Fremont Ford, upstream of the Mud Slough confluence, while Site H is located on the San Joaquin River 200 meters upstream of the confluence of the main branch of the Merced River, downstream of the Mud Slough confluence. Sites C, D, F, and I are monitored by the USFWS while CDFG monitored Sites E, G, and H.

During the WY 2001, biological sampling in Mud Slough was moved from Site I to a new site (Site I2) about 0.5 km upstream of Site I. The new site has a larger, more permanent backwater area.

Sampling Times

Baseline sampling conducted by the USFWS occurred monthly during the spring and summer of 1992 and then less frequently during 1993 and 1994. Baseline sampling by CDFG occurred during the summer and fall of 1993 and then resumed in the spring of 1996. Between 1992 and 1995 sampling by either the CDFG and the USFWS occurred at least once every season. Experience and interagency discussions led to the identification of four sampling times based on historic water use and drainage practices and on seasonal use of wetland resources by fish and wildlife. Biota sampling since 1995 has been synchronized to occur during the months of November, March, June, and August. Since 1996, avian eggs have been collected in May and June.

Due to the 2001 Waste Discharge Requirement Monitoring and Reporting Order, this report covers a fifteen month study period between October 2001 and December 2002.

Statistical Analysis

Student's 2-tail t-tests were used to compare means of concentrations for groups of samples collected at different times at the sampling sites (unpaired samples with unequal variances).

Selenium Hazard Assessment

The protocol proposed by Lemly (1995, 1996) was used to estimate the overall hazard of selenium to the ecosystems affected by the GBP. The implementation of the protocol presented here incorporates data for water from Central Valley Regional Water Quality Control Board and data for sediment from the USBR in addition to biological data collected by the USFWS, CDFG, and CH2M HILL. In accordance with Lemly's protocol, the assessments use the highest (rather than the mean) concentrations of selenium found in each of the ecosystem components (Tables 1 and 5).

Data from the biological sampling in November 1996, shortly after GBP initiation, were excluded from the WY 1997 hazard assessments because temporarily extremely high concentrations of selenium in some fish may have been due to those fish having been flushed out of the previously stagnant, evapo-concentrated SLD. Very high levels of selenium in the water associated with storm flows were not excluded because elevated concentrations persisted long enough (especially in February 1998) potentially to affect the ecosystem adversely.

Concentrations of selenium in fish eggs were estimated from whole-body concentrations using the conversion factor (fish egg selenium = fish whole-body selenium x 3.3) recommended in Lemly (1995, 1996).

In this report, care has been taken to ensure that Lemly index for the area potentially adversely affected by the Grassland Bypass Project incorporates only contaminant levels that are due to this project. Therefore, although Figure 31 displays selenium concentrations in killdeer eggs collected along the San Luis Drain in the Kesterson Reservoir area, those data are not used in the calculation of the Lemly index because of the possibility that some of the most elevated selenium concentrations in eggs are due to killdeer foraging in areas of the Kesterson Reservoir residually contaminated by selenium from Westlands area farms predating this project.

Site E (lower Mud Slough) and the San Joaquin River (SJR) sites (G and H) cannot be rated as to overall hazard of selenium because not all media have been collected to assess these sites. Further confounding the evaluation at these sites is the prevalence of introduced fish species with broad environmental tolerances and the limited catch of invertebrates during WY 1999 and WY 2000.

Departures from the Compliance Monitoring Plan and Quality Assurance Project Plan

To ensure reliable and consistent data, the USFWS and the CDFG followed the procedures specified in the Compliance Monitoring Plan and the Quality Assurance Project Plan (QAPP) with the exceptions listed below.

External quality assurance samples (QAPP Appendix A, Section 7) were not submitted to analytical labs with GBP biological samples before January of 1998. External quality assurance samples are biological materials (e.g. powdered chicken egg, shark liver) with certified concentrations of the analytes of concern (selenium, boron), supplied by third party laboratories. The analyte concentrations in these samples are known to the agencies submitting the samples, but not known to the laboratory doing the analysis. This blind test of laboratory analytical precision supplements the internal quality control procedures of the analytical laboratory. Internal quality control protocols specified in the QAPP (procedural blanks, duplicate samples, and spiked samples) have been followed throughout the history of GBP biological sampling.

The USFWS used stainless steel (rather than Teflon) strainers for sorting small fish (QAPP Appendix A, Section 4.7).

For some species at some locations it has not been practical at some times to collect the full target minimum numbers of individuals and/or mass per sample that are specified in the Compliance Monitoring Plan (Section 4.5.1.4) and the QAPP (Appendix A, Section 4.5).

From 1992 through 1997 all biological samples collected by the USFWS (except bird eggs in 1996 and 1997) were analyzed by Environmental Trace Substance Laboratory at the University of Missouri in accordance with the QAPP (Appendix A, Section 6.1). Bird egg samples collected in 1996 and 1997 were analyzed at Trace Element Research Laboratory (TERL) at Texas A & M University, a USFWS contract laboratory. All biological samples collected in 1998 were analyzed at TERL. TERL is subject to the same performance standards as Environmental Trace Substance Laboratory, therefore, the GBP quality assurance objectives (QAPP Table 1) apply to analytical results from TERL. All biological samples beginning in 1999 have been analyzed at the Water Pollution Control Laboratory of the CDFG in Rancho Cordova, California, after this laboratory was screened and approved by the GBP Quality Control Officer.

Seine net mesh size was increased from 3/16 inch to 1/4 inch after the first two pre-Project collections in 1993 from sampling sites E, G, and H (QAPP Appendix A, Section 4.6). This change in sampling gear resulted in significant declines in catch abundance of smaller forage fish without altering diversity of representative assemblages. Data collected from 1993 sampling efforts at these sites were not included in making quantitative spatial or temporal comparisons between sites unless otherwise noted. At sites C, D, I, and F, 1/8 inch mesh seines were used from 1992 through 1998. Since 1999, a 3/16 inch mesh bag seine has been used at these sites in place of the 1/8 inch mesh bag seine that was previously used by the USFWS.

As discussed earlier, biological sampling in Mud Slough was moved from Site I to Site I2, a new site about 0.5 km upstream with a larger, more permanent backwater area.

Results

Salt Slough (Site F)

Fish (Whole-Body)

Salt Slough is a principal wetland water supply channel from which drainwater has been removed by the GBP. Concentrations of selenium in Salt Slough fish composite samples declined during the first year of operation of the GBP but have stabilized since then at levels well below the concern threshold (Figures 2 and 3), with the exception of March 1998 when concentrations rose in the aftermath of storms that resulted in releases of drainwater into Salt Slough and in June 2001 when the selenium concentration (5.0 mg/kg dry weight)[5] in a single 1.8 gram logperch (Percina caprodes) exceeded the concern threshold for warmwater fish (4 mg/kg). The average of all composite samples of fish at this site during the 15-month period of October 2001 through December 2002 was 2.59 mg/kg (n=57), substantially below the warmwater fish concern threshold (4 mg/kg), significantly below the pre-Project average (6.74 mg/kg, n=77; $p<0.0001$), but not different from the average for the previous year (WY 2001: 2.60 mg/kg, n=51; p=0.89).

Tadpoles

Frog tadpoles (mainly bullfrog, Rana catesbeiana) have been collected only occasionally in the GBP area. Results suggest that in Salt Slough, selenium concentrations in tadpoles, as in fish and invertebrates, declined after implementation of the GBP (Figure 4). A composite sample of four bullfrog tadpoles collected in Salt Slough in August 1999 had about half the selenium concentration (2.6 mg/kg) of a single bullfrog tadpole collected in March 1993 (5.8 mg/kg). Selenium concentrations appeared to rise in the summer of 2000 (2.9 mg/kg in a

5 Calculated from wet weight using average percent moisture of 79.3%

composite sample of three bullfrog tadpoles in June 2000 (7.5 mg/kg in a composite sample of three tadpoles, and 2.3 mg/kg in a single, 19 g frog in August 2000), returned to lower levels in the summer of 2001 (3.8 mg/kg in a single, 0.4 g tadpole in June 2001; 2.5 mg/kg in a composite sample of 13 tadpoles in August 2001), but rose again in the summer of 2002 (5.2 mg/kg in a composite sample of 10 tadpoles in August 2002). The tadpole sample collected in November 2001 (2.9 mg/kg in a composite sample of 4 individuals) was just below the concern level (as diet). However, sample sizes are too small for drawing conclusions about year-to-year trends.

Invertebrates

During the 15-month period of October 2001 through December 2002, selenium concentrations in invertebrates collected from Salt Slough (Figure 5) remained within the range of concentrations associated with no known adverse effects (<3 mg/kg) on animals that eat invertebrates. The mean concentration of selenium in all invertebrate samples collected during this 15-month period (1.6 mg/kg, n=16) was significantly below ($p<0.00001$) the pre-Project mean (4.4 mg/kg, n=27), and significantly below ($p=0.007$) the WY 2001 mean (2.2 mg/kg, n=9).

Mud Slough 0.4 km above SLD Outfall (Site C)

Fish (Whole-Body)

During the 15-month period of October 2001 through December 2002, the average selenium concentration in fish just above the SLD (3.64 mg/kg, n=66) rose significantly from the previous year (WY 2001: 3.0 mg/kg, n=63, $p=0.035$) and was significantly above ($p=0.003$) the pre-Project average at this site (2.78 mg/kg, n=37; Figures 6 and 7). The warmwater fish concern threshold (4 mg/kg; see Table 1) was exceeded by the average selenium concentrations in inland silverside and/or red shiner composite samples in every sampling period from November 2001 through 2002, except June 2002. Elevated average selenium concentrations in some samples at this site may be due to the influence of individual fish swimming upstream from the more contaminated reach of Mud Slough below the discharge of the San Luis Drain.

Tadpoles

At site C, a sample of 16 bullfrog tadpoles (average mass 2.0 g per tadpole) was collected in August 2002. The selenium concentration in this sample (3.28 mg/kg) was in the middle of the range of concentrations in tadpole samples collected previously at this site (Figure 8), above the threshold of concern (3 mg/kg) for dietary effects on birds that may forage on tadpoles. No tadpoles were collected at this site prior to WY1999.

Invertebrates

In the sixth year of operation of the GBP, selenium concentrations in invertebrates at Site C declined even farther below the concern threshold than in previous years, (Figure 9). The average concentration in all invertebrate composite samples in 2002 was 1.34 mg/kg (n=18), significantly below ($p=0.23$) the average of the previous year (1.84 mg/kg, n=14), and significantly below ($p=0.009$) the pre-Project average (1.95 mg/kg, n=15).

Mud Slough 0.2 km below SLD Outfall (Site D)

Fish (Whole-Body)

During the 15-month period of October 2001 through December 2002, at site D, about 200 m below the SLD outfall, the average selenium concentration in small fish (6.19 mg/kg, n=57) decreased significantly (p=0.049) below the average for the previous year (WY 2001: 7.28 mg/kg, n=42), remaining significantly (p<0.0001) above the pre-Project mean (3.83 mg/kg, n=67; Figures 10 and 11). As in previous years, within Water Year 2002, selenium concentrations in fish exhibited significant (p=0.012) seasonal variation (November 2001-March 2002 average: 5.34 mg/kg, n=22; June-August 2002 average: 6.88 mg/kg, n=25). However, the summer increase was less pronounced than in recent previous years (for example, November 2000-March 2001 average: 3.7 mg/kg, n=11; June-August 2001 average: 8.6 mg/kg, n=31, p<0.00001). Though sampling efforts remained generally the same as in previous years, no samples of medium-sized fish were collected from Site D during the fifteen month study period (Figure 11).

Tadpoles

Tadpoles have only be collected occasionally in Mud Slough below the San Luis Drain outfall, and selenium concentrations have always been within the range that is of concern as diet for birds that prey on aquatic vertebrates (3-7 mg/kg). However, during the 15-month period of October 2001 through December 2002, a single 2.3-gram bullfrog tadpole collected in August 2002 at this site had a selenium concentration of 2.37 mg/kg (Figure 12), below the threshold of concern (Figure 12).

Invertebrates

Invertebrates have been relatively scarce at Site D throughout the history of the GBP monitoring program. From October 2001 through December 2002 only three samples of invertebrates (27 backswimmers, 3 red crayfish, and about 200 waterboatmen) could be collected at this site. Average selenium concentration in invertebrate samples (2.52 mg/kg, n=3) during the 15-month period of October 2001 through December 2002 did not change significantly (p=0.224 compared to the previous year (WY 2001: 4.43 mg/kg, n=8; Figure 13).

Mud Slough 1.5 km below SLD Outfall (Site I/I2)

Fish (Whole-Body)

At Site I2, average selenium concentration in fish (8.12 mg/kg, n=63) during the 15-month period from Oct 2001 through December 2002 did not change significantly (p=0.08) compared to the previous water year (WY 2001: 9.24 mg/kg, n=59; Figures 14 and 15). The comparison is confounded by the inclusion of an additional sampling event (Nov. 2002) in the most recent study period and by the inclusion of a single sampling event at the previous Site I in the WY 2001 data (the change of sampling site from Site I to Site I2 occurred in March of 2001; see Beckon et al. 2003). However, a more equal, calendar year comparison also shows no significant difference (p=0.18) between the average selenium concentration in fish at Site I2 (no Site I data included) in 2002 (8.31 mg/kg, n=52) compared to 2001 (9.17 mg/kg, n=64). As at Site D and at Site I in previous years, selenium concentration exhibited a seasonal increase (p=0.013) from early spring (March average 7.55, n=16) to late summer (August average 10.3, n=16). In August 2002 at Site I2, selenium concentrations in all fish samples were elevated well into the toxicity zone for fish as diet for piscivorous birds (>7 mg/kg). All but one sample was above the toxicity threshold for effects on warmwater fish themselves (>9 mg/kg).

As in the previous year, greater bioaccumulation of selenium appeared to occur at I2 compared to Site D. The 15-month (Oct 2001 through Dec 2002) average selenium concentration in all fish samples at Site I2 (8.12 mg/kg) was significantly higher (p=0.004) than the 15-month average at Site D (6.19 mg/kg). This may in part be a real effect due to more efficient bioaccumulation in the backwater conditions at Site I2. However, because Site D is much closer than Site I2 to the Drain discharge point, it is likely that a composite samples of fish and invertebrates collected at Site D include substantial numbers of individuals that have moved downstream from the cleaner reach of Mud Slough above the outfall of the Drain, thereby diluting the average selenium concentrations in the biota at Site D.

<u>Tadpoles</u>

Tadpoles have not been collected at this site.

<u>Invertebrates</u>

Average selenium concentration in all invertebrates collected at Site I2 during the 15-month period of October 2001 through December 2002 (4.51 mg/kg, n=9) was not significantly different (p=0.36) from the previous water year (WY 2001: 5.06 mg/kg, n=13; Figure 16). However, it was significantly higher (p=0.01) than the pre-Project average at Site I (2.65 mg/kg, n=8). Seven of the eight invertebrate samples collected at this site had selenium concentrations above the threshold of concern for birds that would forage on these invertebrates (3 mg/kg). A single sample of zooplankton (a mixture of thousands of microscopic invertebrates, mainly Daphnia) collected at this site in November 2002 had a selenium concentration of 4.82 mg/kg, well above the selenium concentration in the single sample of more than 200 waterboatmen collected at the same time at the same site (2.16 mg/kg). This suggests that microscopic invertebrates may represent an even greater risk to the aquatic and aquatic-dependent food webs than the larger water-column invertebrates (waterboatmen and backswimmers) that have been the focus of water-column invertebrate monitoring in this project.

Lower Mud Slough and San Joaquin River Sites

<u>Mud Slough at Highway 140 (Site E)</u>

Site E is located in lower Mud Slough downstream from Sites D and I2 but upstream from the confluence of Mud Slough with the San Joaquin River. This site represents the lower reach of the Slough that is affected by the operation of the Project. At this point along Mud Slough, within the flood plain of the San Joaquin River, flows are slower and more spread out, and flood waters of the San Joaquin River periodically back up into slough, providing some flushing. Selenium in whole body fish and invertebrate samples collected at this site in WY 1999, 2000 and 2001 and the fifteen month study period confirm the trend of increasing concentrations that is evident at Sites D, I, and I2.

<u>Fish (Whole-Body)</u>

The concentration of selenium in composite samples of whole-body mosquitofish (*Gambusia affinis*) collected during the fifteen month study period ranged from 8.8 to 14.8 mg/kg (dry weight), with six of seven samples exceeding the toxicity threshold (9 mg/kg dry weight) in June, August, and December 2002 (Figure 17). The average selenium concentration of all fourteen samples of whole-body fish collected from this site during the fifteen month study period was 11.6 mg/kg.

The average concentration of selenium in six composite samples of wholebody mosquitofish collected during WY 2002 was 11.04 mg/kg (dry weight). This was not significantly different from samples collected during WY 2001 (9.22 mg/kg dry weight, n=12, p=0.123), but is significantly higher than the average concentration of samples collected during WY 2000 (6.77 mg/kg dry weight, n=12, p=0.002) and the average pre-project concentration of 2.5 mg/kg dry weight (n=12, p<0.000).

Invertebrates

Crayfish were not difficult to catch at this site during the fifteen month study period. Six composite samples of crayfish collected at this site during November 2001 and March 2002 had selenium concentrations within the concern range (3 - 7 mg/kg dry weight) for invertebrates (Figure 18). Two composite samples collected during August and December 2002 exceeded the toxicity threshold of 9 mg/kg dry weight.

The average concentration of selenium in all six crayfish samples collected during WY 2002 was 5.96 mg/kg (dry weight). This concentration was the same as the previous two water years, but significantly higher than the average selenium concentration in crayfish caught at this site before 1996 (μ=1.72 mg/kg dry weight, n=15, p=0.009).

The concentration of selenium in waterboatmen collected from this site during March 2002 was 4.1 mg/kg (dry weight), above the 3 mg/kg (dry weight) concern threshold. In prior water years, annual samples of waterboatmen were below the 3 mg/kg concern threshold.

San Joaquin River at Fremont Ford (Site G)

Site G is located at Fremont Ford on the San Joaquin River upstream of the Mud Slough confluence. This site represents the reach of the San Joaquin River that no longer receives agricultural drainwater from the Grassland Drainage Area as a result of the GBP.

Fish (Whole-Body)

Similar to the first five years of GBP operation, selenium concentrations in composite samples of fish collected from this site continued to reflect removal of selenium-laden drain water. Selenium concentrations in composite samples of whole-body mosquitofish collected during the fifteen month study period ranged from 1.17 to 1.89 mg/kg (dry weight), remaining well below the concern threshold (4 mg/kg dry weight) for warmwater fish (Figure 19). Average selenium concentration for all mosquitofish collected in the fifteen month study period was 1.62 mg/kg (dry weight) (n=15).

The average concentration of selenium in twelve composite samples of mosquitofish collected during WY 2002 was 1.64 mg/kg (dry weight). This was less than the previous year (WY 2001, μ=1.99, n=12, p=0.001), and significantly less than the pre-project average concentration of selenium of 4.79 mg/kg (dry weight) measured in fifteen samples. Selenium concentrations in whole-body mosquitofish have consistently been within or below the Concern range (4 - 9 mg/kg dry weight) since the GBP began September 1996.

Invertebrates

Selenium concentrations in all invertebrates collected from this site during the fifteen month study period were less than all previous years since project operations began (Figure 20). The average concentration of selenium in nine composite samples of crayfish collected during the fifteen month study period was 1.21 mg/kg (dry weight). The selenium concentrations ranged from 0.92 to 2.36 mg/kg (dry weight), remaining below the 3 mg/kg (dry weight) threshold of concern for invertebrates as prey items.

The average concentration of selenium in seven composite samples of red crayfish caught during WY 2002 was 1.02 mg/kg (dry weight). This was not significantly different than the average concentration of selenium in nine crayfish samples caught at this site during WY 2001 (μ=1.48 mg/kg, p=0.047). The WY 2002 average selenium concentration was significantly greater than that for WY 2000 (μ=0.42, n=8, p=0.000). However, the average selenium concentration of all samples collected during WY 2002 was significantly less than the pre-project level of 3.5 mg/kg dry weight (n=9, p=0.001).

Similar to crayfish, the concentration of selenium in all samples of waterboatmen collected from this site during WY 2002 continued to be well below the 3 mg/kg (dry weight) concern threshold, with an average selenium concentration of 1.4 mg/kg (dry weight); All samples of waterboatmen have consistently remained below the concern threshold during all water years since Project operations began September 1996.

San Joaquin River Below Mud Slough (Site H)

Site H is located at Hills Ferry on the San Joaquin River about two miles downstream of the Mud Slough confluence. This site represents the reach of the San Joaquin River most strongly influenced by agricultural drain water discharged by the GBP. One of the environmental commitments of the GBP is that it will not worsen water quality in the San Joaquin River. For practical reasons of year-round accessibility, the site was located just upstream of the Merced River confluence; Merced River waters have relatively low concentrations of selenium. It is possible that some of the fish and invertebrates collected at Site H have moved into this area after foraging within the Merced River and other less contaminated reaches of the San Joaquin River.

Additionally, seasonally high flows in the Merced River can enter the San Joaquin River upstream of Site H, temporarily diluting the load of contaminants there. Due to these confounding influences on selenium body burdens, selenium concentrations in fish and invertebrate tissues collected at this site may not be well correlated with water concentrations of selenium at this site.

Fish (Whole-Body)

Selenium concentrations in fifteen composite samples of whole-body mosquitofish collected during March and December 2002 were above the 4 mg/kg (dry weight) concern threshold for warmwater fish (Figure 21). The average of all samples collected during the fifteen month study period (μ = 4.12 mg/kg)

The average concentration of selenium in twelve composite samples of wholebody mosquitofish collected form this site during WY 2002 was 3.82 mg/kg (dry weight). This was not significantly different than the previous water year (μ=3.75 mg/kg, n=9, p=0.749). Despite this, selenium concentrations in composite whole-body fish samples throughout the five years of GBP operation have generally remained below the 4 mg/kg (dry weight) concern threshold and are not significantly different from selenium concentrations in fish collected before the GBP began in 1996 (μ=3.78, n=21, p=0.924).

Invertebrates

Selenium concentrations in nine composite samples of red crayfish collected from this site during the fifteen month study period ranged from 1.31 mg/kg to 5.08 mg/kg (dry weight), with an average of 2.69 mg/kg, which is slightly below the 3 mg/kg (dry weight) concern threshold associated with known adverse effects on higher order consumers (Figure 22). The concentration of selenium in one composite sample of water boatmen, collected March 2002, was 2.73 mg/kg (dry weight), similar to WY 2001.

The average concentration of selenium in eight composite samples of red crayfish caught during WY 2002 was 2.40 mg/kg (dry weight). This average was not significantly different than the previous water year (μ=3.34, n=3, p=0.053) or from the concentration of selenium measured in nine samples collected before the project began in 1996 (μ=2.08 mg/kg, p=0.541).

Fish Communities Assessment

Fish communities assessments are conducted to describe fish assemblages based on species richness, abundance and community structure. Fish populations were sampled in Mud Slough at Highway 140 (Site E), San Joaquin River at Fremont Ford (Site G), and San Joaquin River below Mud Slough (Site H). Fish assemblages from these sites were compared both spatially and temporally to see if conditions for fish species in the San Joaquin River improved and conditions in Mud Slough degraded. We sampled in August and November 1993, March, June, and August/September of the years 1996 – 1999, November 2001, and December 2002. We did not sample during November 2000. As the Grassland Bypass Project began operation in September 1996, this sampling schedule provided a before-and-after picture of the fish communities at these sites. Only data collected with standardized sampling methodologies and effort were analyzed.

Table 3 is a compilation of the 34 fish species, represented by 20,104 individuals, that have been collected at these sites during five pre-project and eighteen post-Project sampling events. Ten species of native fish were caught, representing only three percent of the catch by number (n = 512).

Only four native species were caught during November 2001 and December 2002 at the three sites: Pacific staghorn sculpin (Leptocottus armatus, n= 74), Sacramento sucker (n=4), Sacramento splittail (n=3), and Sacramento blackfish (n=2). The fish screen at Site H prevents salmon from moving upstream to the sampling sites for this project.

Pacific staghorn sculpin were the most abundant native fish throughout the study. The most common non-native fish are mosquitofish, inland silversides, fathead minnow, and carp.

No time trends are apparent in fish species assemblages during the period 1993 to 2002 at Sites E, G, and H (Figures 23-25). Omnivores were dominant at Site E and invertivores were dominant at Sites G and H in the San Joaquin River. No time trend is evident in total anomalies for the various groups of fishes at each site (Figure 26).

During September and October 1997, about one year after the reopening of the SLD, Saiki (1998) sampled fish at 13 sites in the Grassland area. These sites correspond to locations he had surveyed more than a decade earlier (Saiki 1986). Some of his sample sites were the same as, or close to, GBP monitoring sites, but others were located in areas not monitored by the GBP. The SLD was the only site in the area that lacked bluegill and goldfish, and overall, fewer species of fish were found in the SLD than at any other site. However, Saiki did not find any significant difference in community structure related to the proportion of drainwater present. To explain this, he noted that all waterways in the area are overwhelmingly dominated by introduced species having broad environmental tolerances. Saiki's findings are consistent with those of the GBP biological monitoring program.

After 6 years of Project operation, no clear pattern of temporal or geographic variation in fish community structure attributable to the Project has emerged. However, current methods of assessing fish species assemblages may lack the power to detect all but the most pronounced alterations in community structure.

Assessment of Risk to Public Health from Consumption of Fish

During the first five years of GBP operation, samples of carp muscle tissue collected from Site E were below the 2 mg/kg health screening level for selenium, except for samples collected in September 1997 and August 1998. The concentration of selenium in eleven composite samples of carp caught between March 1999 and August 2001 ranged from 0.84 – 1.68 mg/kg (wet weight). These concentrations are comparable to those in four composite samples caught before the GBP began (0.61 – 1.25 mg/kg wet weight). During the fifteen month study period, the average concentration of selenium in samples of carp collected in November 2001 and August 2002 exceeded the 2 mg/kg health screening level. The average concentration of selenium in carp tissue collected in March, June, and December 2002 did not exceed the health screening level (Figure 27).

The concentration of selenium in carp collected at Site E during the fifteen month study period ranged from 0.51 to 2.73 mg/kg (wet weight, n=15). Four composite samples collected in November 2001 and August 2002 exceeded the 2 mg/kg (wet weight) selenium health screening level (Figure 27).

The average concentration of selenium in twelve carp muscle tissue sampled during the Water Year 2002 was 1.67 mg/kg (wet weight). This average was significantly different than the average from the previous water year (μ=1.21 mg/kg, n=9, p=0.050) and from the average of eleven samples collected prior to the beginning of the project in 1996 (μ=0.74 mg/kg, p=0.001).

The concentration of selenium concentrations in carp fillets collected at Sites G (μ=0.51 mg/kg wet wt, n=15) and H (μ=0.74 mg/kg wet weight, n=15) on the San Joaquin River have remained consistently below the 2 mg/kg health screening level throughout all five years of GBP operations (Figures 28 and 29).

Selenium in Plants

Composite samples of plant material that provides preferred forage for waterfowl (seed heads) have been collected in late summer for several years, but funding has only been adequate to analyze some of these materials for selenium in the last two years (Figure 30). In WY 2002, the highest selenium concentrations found in water-side plants were from samples collected along Mud Slough downstream of the San Luis Drain (Sites D and I2). All samples were well below the threshold of concern for reproductive effects on waterfowl due to dietary exposure (3 mg/kg) except a composite sample of swamp timothy seed heads (3.5 mg/kg) collected from the banks of Mud Slough below the San Luis Drain outfall (Site D). The selenium concentration in samples of bullrush sedge, cattail, and swamp timothy collected at sites C, D, F and I2 in August 2002 were all below the analytical reporting limit of 0.20 mg/kg, dry weight. These data suggest that birds in this area are generally at greater risk due to eating invertebrates and fish than from eating plants.

The concentrations of selenium in knotgrass (Paspalum disthum) seed heads collected by CDFG at Sites E, G, and H were below the 3 mg/kg (dry weight) threshold of concern. The average concentration of selenium in three composite samples of seeds collected during August 2002 at Site E was 0.55 mg/kg dry weight. This average is significantly different from the average of seed samples collected before the GBP began in 1996 ($\mu=0.30$, $n=3$, $p=0.031$).

The average concentration of selenium in seed collected at Site G was 0.03 mg/kg dry weight. This average was significantly less than the average selenium concentration in seed collected before the GBP began ($\mu=0.20$ mg/kg dry weight, $p=0.000$).

The average concentration of selenium in seed collected at Site H was 0.15 mg/kg dry weight. This average was not significantly different than the average selenium concentration in seed collected before the GBP began ($\mu=0.23$ mg/kg dry weight, $p=0.293$).

Selenium in Bird Eggs

In 2002, a single egg was randomly collected and analyzed from each of 13 bird nests in the Grassland area, and, for comparison, from one mallard duck nest on the San Joaquin River National Wildlife Refuge (Figure 31). Species sampled included killdeer, American avocet, wood duck, barn swallow, cliff swallow, and starling. The selenium concentrations in all eggs collected in 2002 were within the "no effect" range of concentrations (<6 mg/kg). Selenium concentrations in eggs analyzed from the Mud Slough area (geometric mean 2.38 mg/kg, $n=10$) were not significantly different ($p=0.56$, t-test performed on log-transformed concentrations) from those analyzed from the Salt Slough area (geometric mean 2.14 mg/kg, $n=4$) in 2002.

Aquatic Hazard Assessment of Selenium

To provide an estimate of ecosystem-level effects of selenium, Lemly (1995, 1996) developed an aquatic hazard assessment procedure that sums the effects of selenium on various ecosystem components to yield a single characterization of overall hazard to aquatic life. Lemly's procedure applied to Mud Slough downstream of the SLD outfall indicated that the hazard to aquatic life in the affected portion of Mud Slough continued to be "high" in WY 2002 (Table 3).

In the Salt Slough area, the Lemly index rose from "low" in WY 2000 to “moderate” in WY 2001 and back to low in WY 2002 (Table 3). Because the Lemly index is based on maximum concentrations, it is highly sensitive to data “outliers”. A Lemly index was not determined for San Joaquin River sites due to lack of sufficient sample of invertebrates and because bird eggs, one component of the index, were not sampled there.

Boron in Plants

Samples of seed heads from plants (knotgrass, smartweed, swamp timothy, bullrush sedge) collected in August 2002 from Sites C, D, E, I2, F, G, and H were analyzed for boron.

At Site C, one of two samples (12.5, 47.5) exceeded the threshold of concern for boron in plants as diet (30 mg/kg, Table 2). One of three samples collected at Sites D and I2 were above the threshold of concern (Site D: 13.7, 64.2 mg/kg ; Site I2: 28.9). At Site E all samples exceeded the threshold of concern (74.5, 119, and 73.3 mg/kg). At Site F, the single sample analyzed was slightly above (30.6 mg/kg) the threshold of concern.

The concentration of boron in knotgrass seedheads (Paspalum distichum) collected at Site G on the San Joaquin River was 16.1 mg/kg (n=3), below the threshold of 30 mg/kg. The concentration of boron in knotgrass seedheads collected at Site H was 44.4 mg/kg which is above the threshold of concern.

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Table 1. Recommended Ecological Risk Guidelines for Selenium Concentrations.

Medium	Effects on	Units	No Effect	Concern	Toxicity
Water (total recoverable selenium)	fish and bird reproduction	µg/L	< 2	2 -- 5	**> 5**
Sediment	fish and bird reproduction	mg/kg (dry weight)	< 2	2 -- 4	**> 4**
Invertebrates (as diet)	bird reproduction	mg/kg (dry weight)	< 3	3 -- 7	**> 7**
Warmwater Fish (whole body)	fish growth/condition/survival	mg/kg (dry weight)	< 4	4 -- 9	**> 9**
Avian egg	egg hatchability (via foodchain)	mg/kg (dry weight)	< 6	6 -- 10	**> 10**
Vegetation (as diet)	bird reproduction	mg/kg (dry weight)	< 3	3 -- 7	**> 7**

Notes:

1/ These guidelines, except those for avian eggs, are intended to be population based. Thus, trends in means over time should be evaluated. Guidelines for avian eggs are based on individual level response thresholds (e.g., Heinz, 1996; Skorupa, 1998)

2/ A tiered approach is suggested with whole body fish being the most meaningful in assessment of ecological risk in a flowing system.

3/ The warmwater fish (whole body) concern threshold is based on adverse effects on the survival of juvenile bluegill sunfish experimentally fed selenium enriched diets for 90 days (Cleveland et al., 1993). It is the geometric mean of the "no observable effect level" and the "lowest observable effect level."

4/ The toxicity threshold for warmwater fish (whole body) is the concentration at which 10% of juvenile fish are killed (DeForest et al., 1999).

5/ The guidelines for vegetation and invertebrates are based on dietary effects on reproduction in chickens, quail and ducks (Wilber, 1980; Martin, 1988; Heinz, 1996).

6/ If invertebrate selenium concentrations exceed 6 mg/kg then avian eggs should be monitored (Heinz et al., 1989; Stanley et al., 1996).

Table 2. Recommended Ecological Risk Guidelines for Boron Concentrations.

Medium	Effects on	Units	No Effect	Concern	Toxicity
Water	fish (catfish and trout embryos)	mg/L	< 5	5 -- 25	**> 25**
Water	invertebrates (*Daphnia*)	mg/L	< 6	6 -- 13	**> 13**
Water	vegetation (crops and aquatic plants)	mg/L	< 0.5	0.5 -- 10	**> 10**
Waterfowl diet	duckling growth	mg/kg (dry weight)		> 30	
Waterfowl egg	embryo mortality	mg/kg (dry weight)	<1	> 10	**>30**

Notes:

1/ Water guidelines for invertebrates are based on the "no observed adverse effects level" and "lowest observed adverse effects level" for *Daphnia magna* (Lewis and Valentine 1981; Gersich 1984).

2/ Waterfowl diet guidelines are based on mallard ducks (Smith and Anders 1989).

3/ The waterfowl egg no effect level is based on poultry data from Romanoff and Romanoff (1949) and San Joaquin Valley field data for reference sites (R. L. Hothem and Welsh; J. P. Skorupa et al.).

4/ The waterfowl egg concern and toxicity thresholds are based on Smith and Anders (1989), Stanley et al. (1996), and the "order-of-magnitude rule of thumb" (toxicity at about 10 times background concentrations).

5/ The US Environmental Protection Agency's suggested no adverse response level for drinking water is 0.6 mg/L.

Table 3. Fishes collected from Grassland Bypass Project Stations E, G, and H in decreasing order of numerical abundance. August 1993 - December 2002

Species Common name, *Scientific name*	Number Collected	Origin	Trophic Classification	Tolerance to environmental degradation	native
Mosquitofish, *Gambusia affinis*	14,368	Introduced	I	T	0
Inland silverside, *Menidia beryllina*	3,370	Introduced	I	M	0
Carp, *Cyprinus carpio*	2,505	Introduced	O	T	0
Fathead minnow, *Pimephales promelas*	2,184	Introduced	O	T	0
Red shiner, *Cyprinella lutrensis*	1,318	Introduced	O	T	0
White catfish, *Ameiurus catus*	1,298	Introduced	I/P	T	0
Bluegill, *Lepomis macrochirus*	866	Introduced	I	T	0
Threadfin shad, *Dorosama petenese*	513	Introduced	I	M	0
Largemouth bass, *Micropterus salmoides*	454	Introduced	P	T	0
Goldfish, *Carassius auratus*	404	Introduced	O	T	0
Green sunfish, *Lepomis cyanellus*	382	Introduced	I/P	T	0
Redear sunfish, *Lepomis microlophus*	279	Introduced	I	M	0
Channel catfish, *Ictalurus punctatus*	254	Introduced	I/P	M	0
Sacramento blackfish, *Orthodon microlepidotus*	219	Native	O	T	219
Warmouth, *Lepomis gulosus*	215	Introduced	I	M	0
Splittail, *Pogonichthys macrolepidotus*	111	Native	O	M	111
Bigscale logperch, *Percina macrolepida*	101	Introduced	I	T	0
Pacific staghorn sculpin, *Leptocottus armatus*	74	Native	I/P	M	74
Black crappie, *Pomoxis nigromaculatus*	57	Introduced	I/P	M	0
Brown bullhead, *Ameiurus nebulosus*	40	Introduced	I/P	T	0
Smallmouth bass, *Micropterus* dolomieui	37	Introduced	I/P	M	0
Spotted bass, *Micropterus punctulatus*	37	Introduced	P	M	0
Striped bass, *Morone saxatilis*	30	Introduced	P	M	0
Sacramento sucker, *Catostomus occidentalis*	29	Native	O	M	29
Prickly sculpin, *Cottus asper*	28	Native	I	M	28
Chinook salmon, *Oncorhynchus tshawytscha*	26	Native	I	I	26
Sacramento pikeminnow, *Ptychocheilus* grandis	21	Native	I/P	M	21
Black bullhead, *Ameiurus melas*	14	Introduced	I/P	T	0
American shad, *Alosa sapidissima*	13	Introduced	I	M	0
Golden Shiner, *Notemigonus crysoleucas*	11	Introduced	I	M	0
Bullfrog, *Rana catesbeiana*	10	Introduced	O	T	0
White crappie, *Pomoxis annularis*	10	Introduced	I/P	T	0
Red crayfish, *Procambarus clarkii (Scapulicambar*	6	Introduced	O	T	0
Hitch, *Lavinia exilicauda*	4	Native	O	M	4
Tule perch, *Hysteocarpus traski*	4	Native	I	I	4
Pumpkinseed, *Lepomis gabbosus linaeas*	2	Introduced	I	M	0
Riffle sculpin, *Cottus gulosus*	1	Native	I	M	1
Total	29,295				517
Data Source: California Department of Fish and Game					2%

Notes:

Trophic Classification: O - omnivore
I - invertivore
P - piscivore
I/P - invertivore/piscivore

Tolerance to environmental degradation: I - intolerant
M - moderately tolerant
T - tolerant

Table 4. Aquatic Hazard Assessment of Selenium in Mud and Salt Slough

		BEFORE PROJECT 1995 - Sept. 1996			GRASSLAND BYPASS PROJE WY1997			WY1998			WY1999			
	Units	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration
Mud Slough below Drain outfall														
Water	µg/L	19	high	5	80	high	5	104	high	5	51	high	5	66
Sediment	µg/g	0.4	none	1	0.8	none	1	2.0	low	3	4.8	high	5	4.4
Invertebrates	µg/g	1.6	none	1	3.3	low	3	11.0	high	5	7.0	high	5	15.3
Fish eggs	µg/g	14.2	moderate	4	56.1	high	5	34.2	high	5	39.6	high	5	46.5
Bird eggs	µg/g	3.1	minimal	2	4.4	minimal	2	6.6	low	3	10.0	low	3	5.1
TOTAL HAZARD SCORE			**Moderate**	**13**		**High**	**16**		**High**	**21**		**High**	**23**	
Salt Slough														
Water	µg/L	38	high	5	3	moderate	4	5	high	5	2	minimal	2	2
Sediment	µg/g	0.8	none	1	0.9	none	1	2.1	low	3	0.9	none	1	0.7
Invertebrates	µg/g	4.7	moderate	4	2.6	minimal	2	3.2	low	3	2.8	minimal	2	2.7
Fish eggs	µg/g	28.1	high	5	17.8	moderate	4	12.9	moderate	4	11.2	moderate	4	14.5
Bird eggs	µg/g	5.2	low	3	3.6	minimal	2	3.7	minimal	2	2.7	none	1	4.9
TOTAL HAZARD SCORE			**High**	**18**		**Moderate**	**13**		**High**	**17**		**Low**	**10**	

Hazard Scale:			**TOTAL HAZARD SCORE**	
	5	high	**16 - 25**	**High**
	4	moderate	**12 - 15**	**Moderate**
	3	low	**9 - 11**	**Low**
	2	minimal	**6 - 8**	**Minimal**
	1	none	**0 - 5**	**None**

Notes: Table prepared by US Fish and Wildlife Service, Sacramento.
(*) October 1, 2001 - December 31, 2002.

Table 5. Maximum contaminant concentration data used for the Lemly Index (Table 4) for the period October 1, 2001 to December 31, 2002.

Mud Slough (San Luis Drain, Sites D, I, and I2)

Media	Sample Date	Location	Value	Sample Type	Sample Size	Data Source
Water	26-Apr-01	Site D	51 µg/L	weekly grab		CVRWQCB
Sediment	14-Nov-00	Site I	3.5 µg/g	0 - 3 cm		USBR
Invertebrates	21-Aug-01	Site I2	7.1 µg/g	waterboatmen	n=ca 200	US FWS I2B01AUG19
Fish eggs (*)	21-Aug-01	Site I2	16.6 µg/g whole- body Se x 3.3 = 54.8 µg/g in eggs	mosquitofish wholebody	n=25	US FWS I2B01AUG21
Bird eggs	25-Apr-01	SLD	7 µg/g	black phoebe	n=1	US WS SLD01APR02

(*) fish egg selenium = fish wholebody selenium x 3.3

Salt Slough (Site F)

Media	Sample Date	Location	Value	Sample Type	Sample Size	Data Source
Water	14-Mar-01	Site F	2 µg/L	weekly grab sample		CVRWQCB
Sediment	14-Mar-01	Site F	0.8 µg/g	whole core		USBR
Invertebrates	21-Mar-01	Site F	2.7 µg/g	Red crayfish	n=6	US FWS F01MAR07
Fish eggs (*)	21-Mar-01	Site F	3.8 µg/g whole- body Se x 3.3 = 12.5 µg/g in eggs	logperch	n=1	US FWS F01MAR06
Bird eggs	8-May-01	Site F	4 µg/g	black-necked stilt	n=1	US FWS SL01MAY01

(*) fish egg selenium = fish wholebody selenium x 3.3

Figure 1. Grassland Bypass Project biota monitoring sites

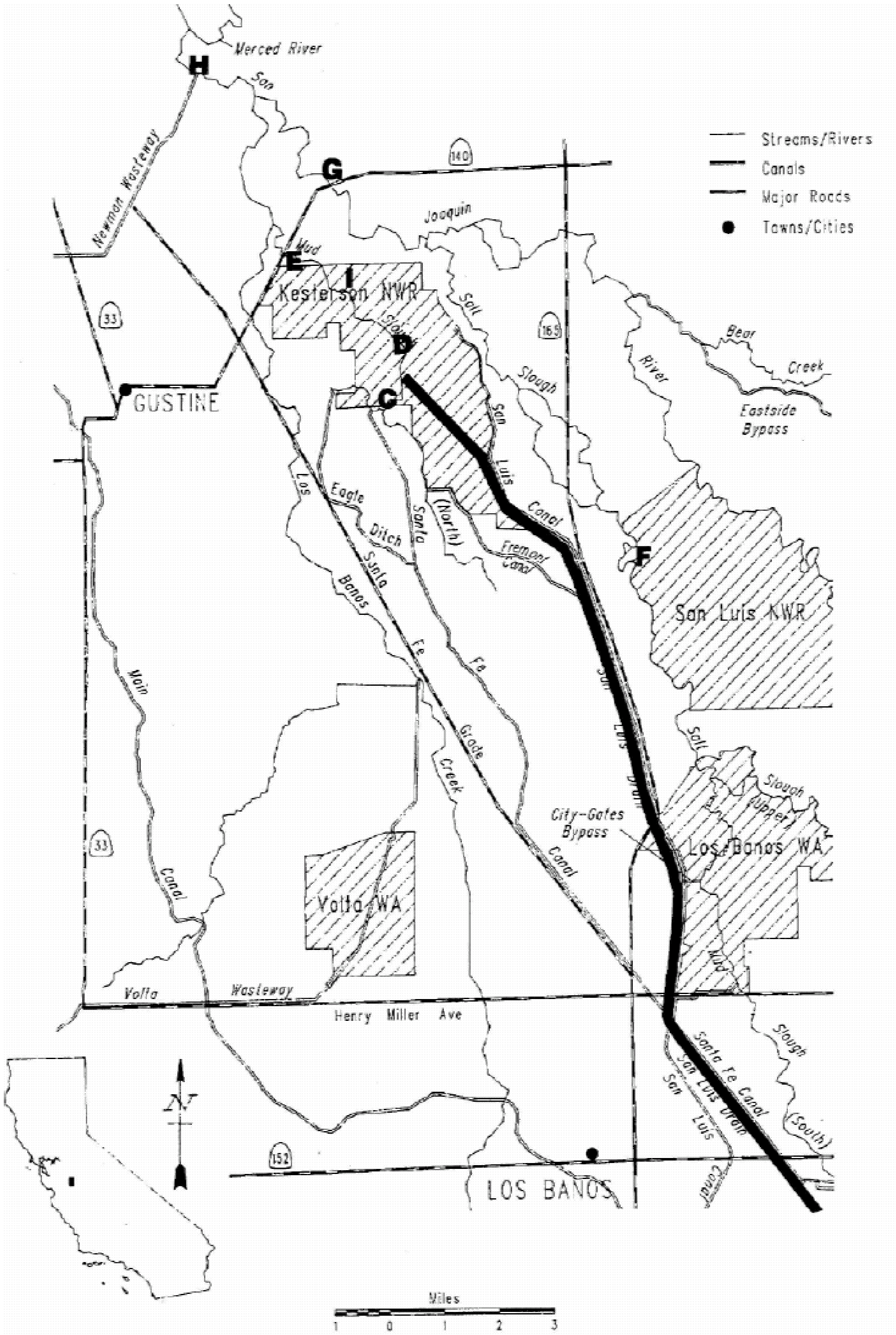


**Figure 2. Selenium in small fish in Salt Slough (Site F).
Each bar represents an average of composite samples.**

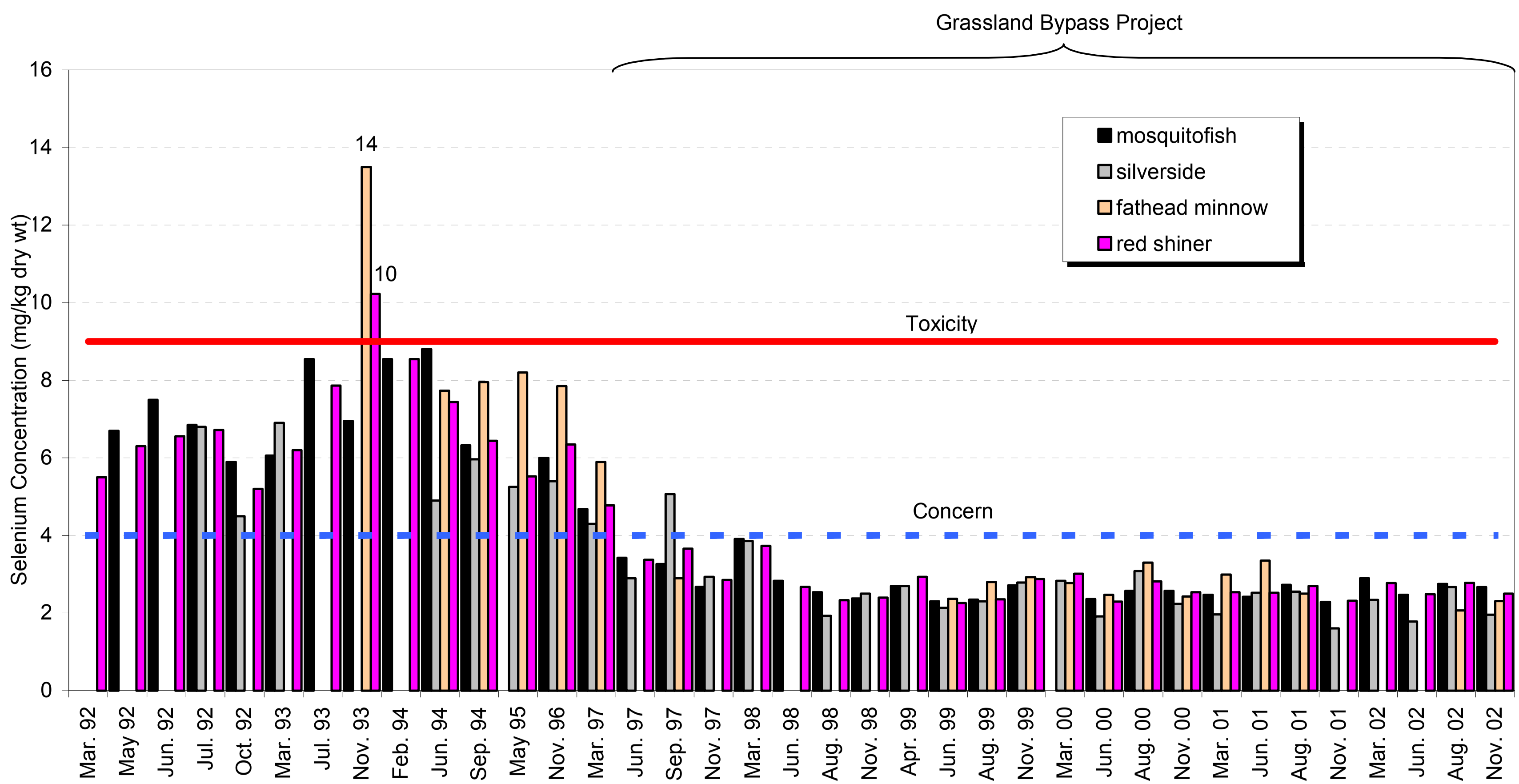


Figure 3. Selenium in medium-size fish in Salt Slough (Site F).

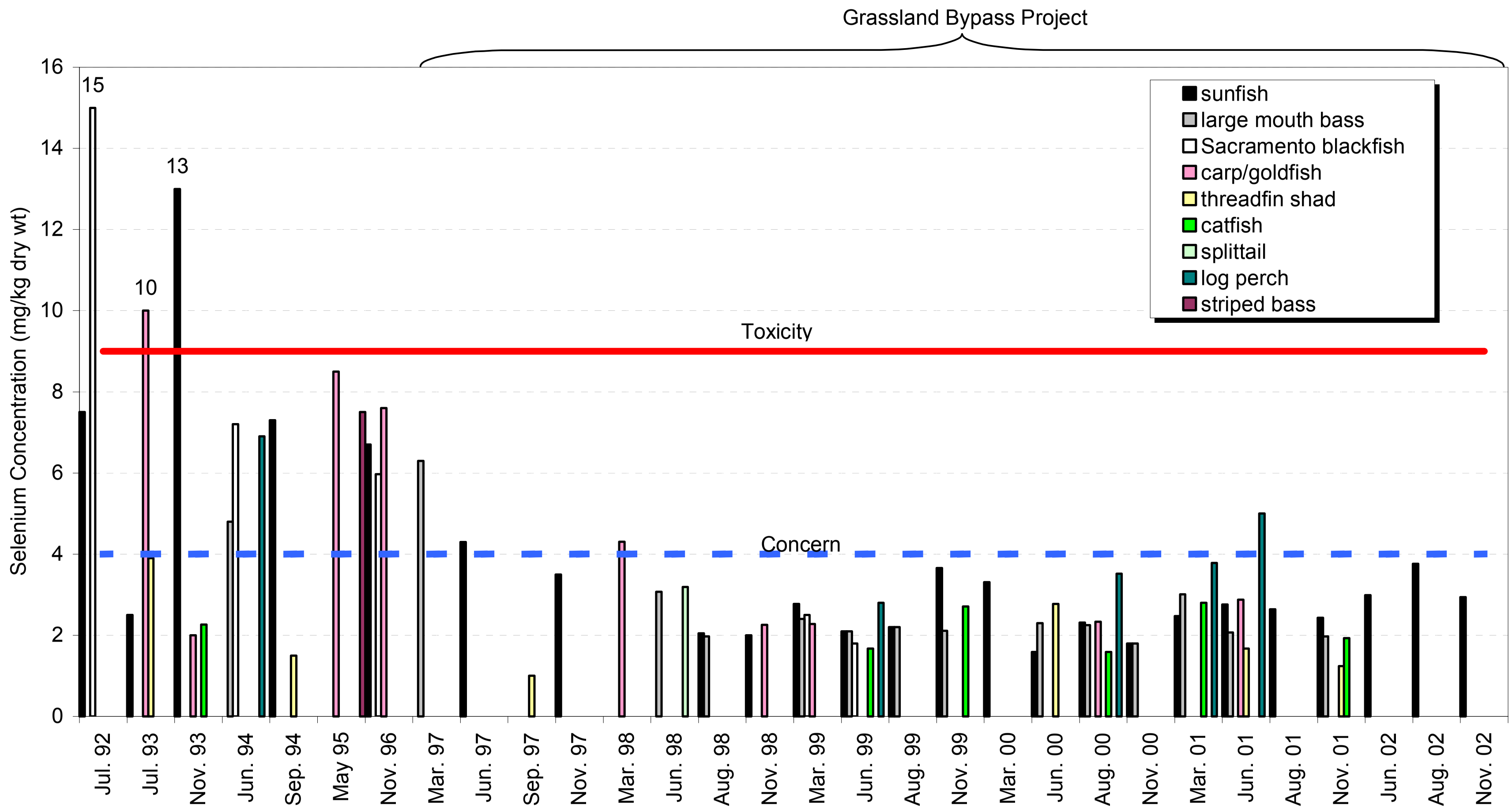


Figure 4. Selenium in bullfrog tadpoles in Salt Slough (Site F).

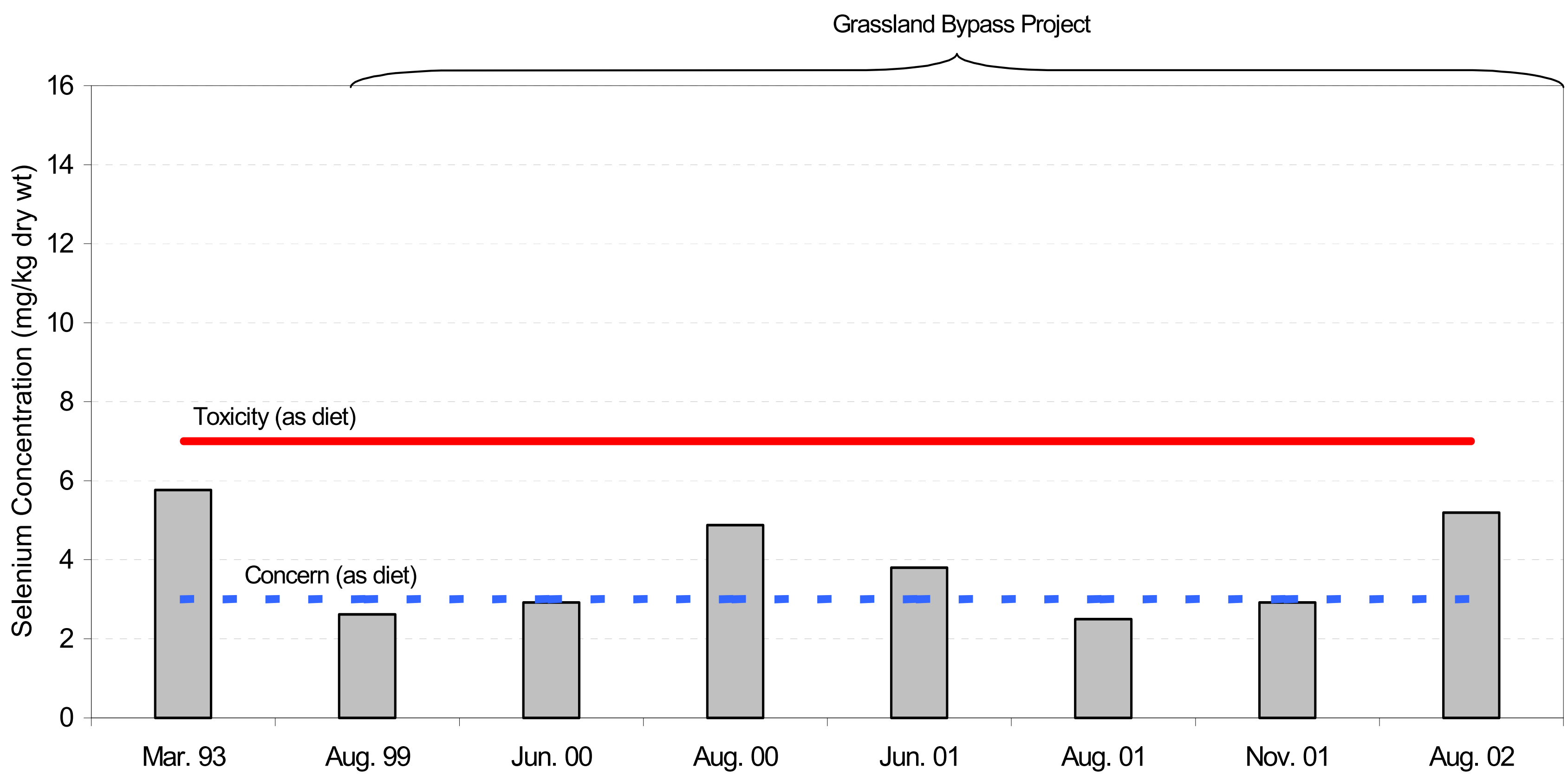


Figure 5. Selenium in invertebrates in Salt Slough (Site F).

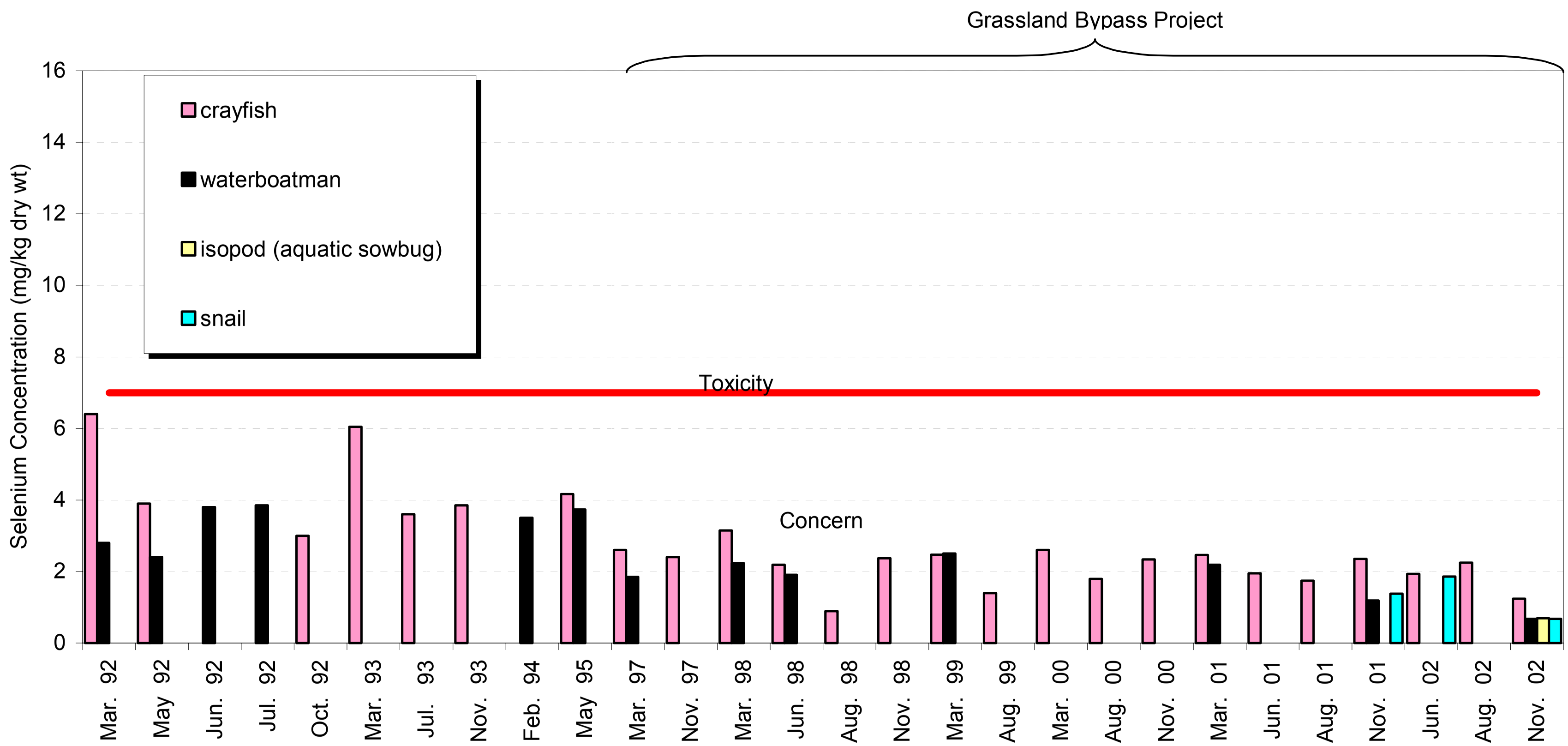


Figure 6. Selenium in small fish in Mud Slough above the San Luis Drain discharge (Site C).

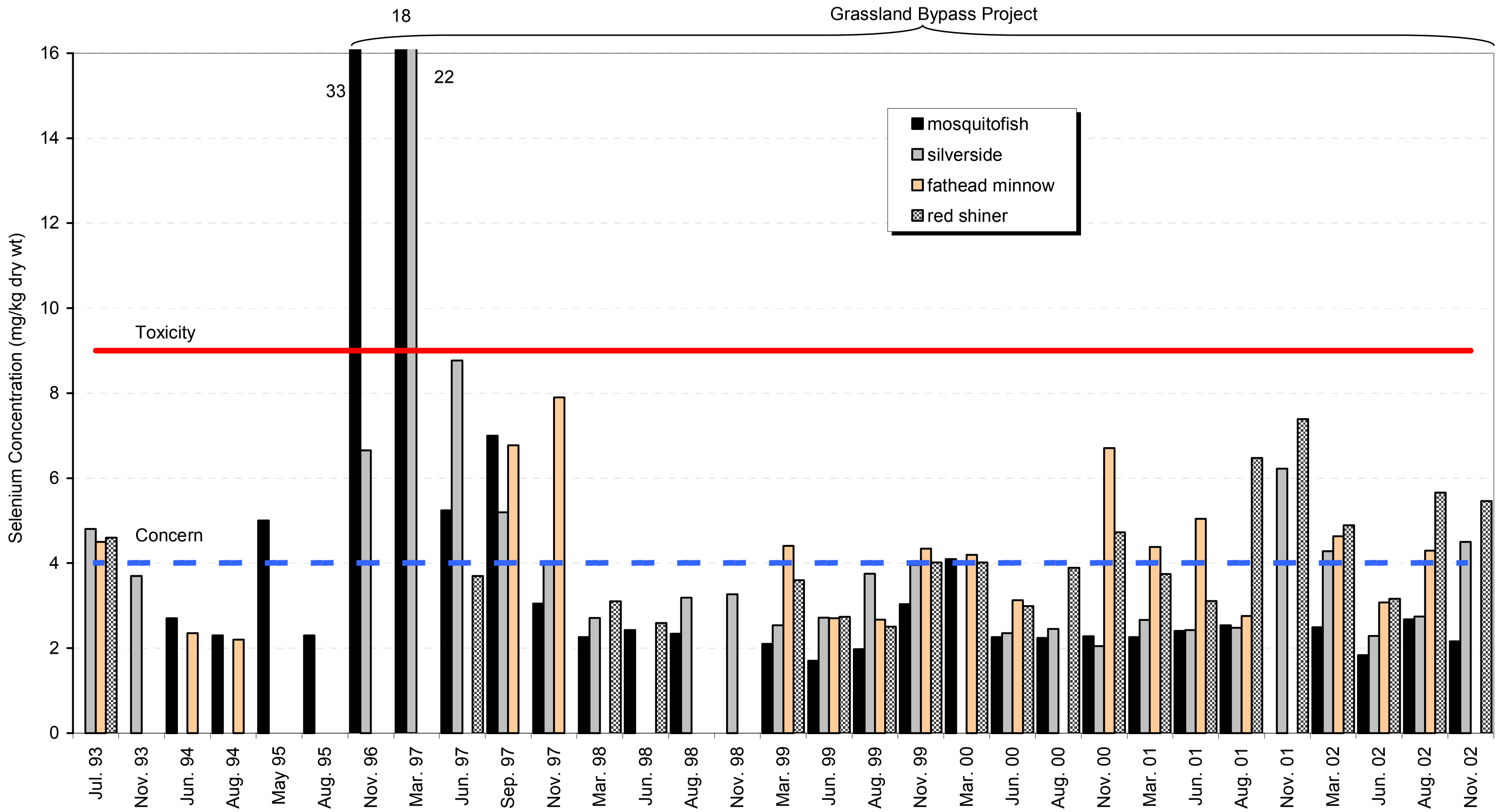


Figure 7. Selenium in medium-size fish in Mud Slough above the San Luis Drain discharge (Site C).

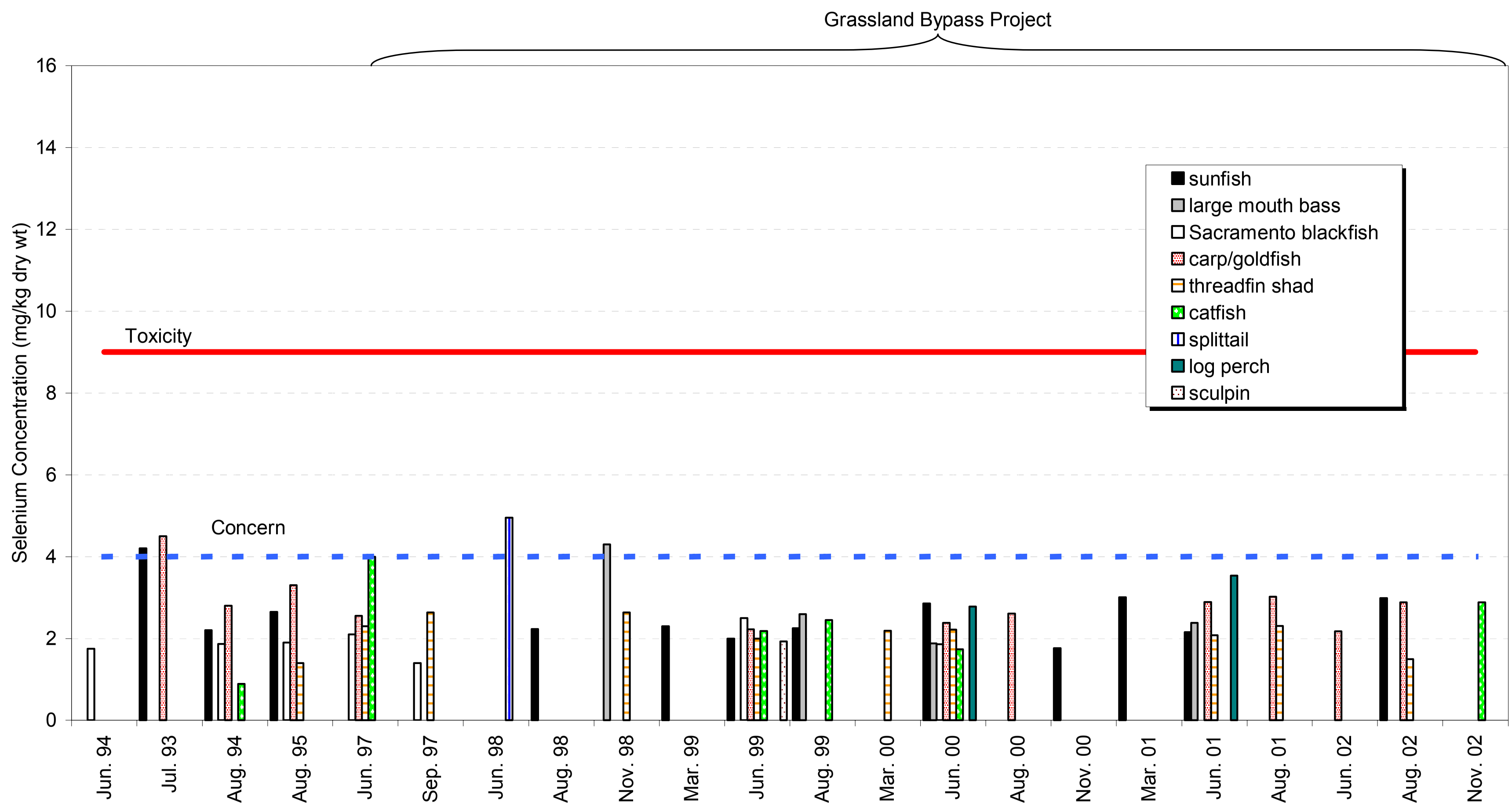


Figure 8. Selenium in bullfrog tadpoles in Mud Slough above the San Luis Drain discharge (Site C).

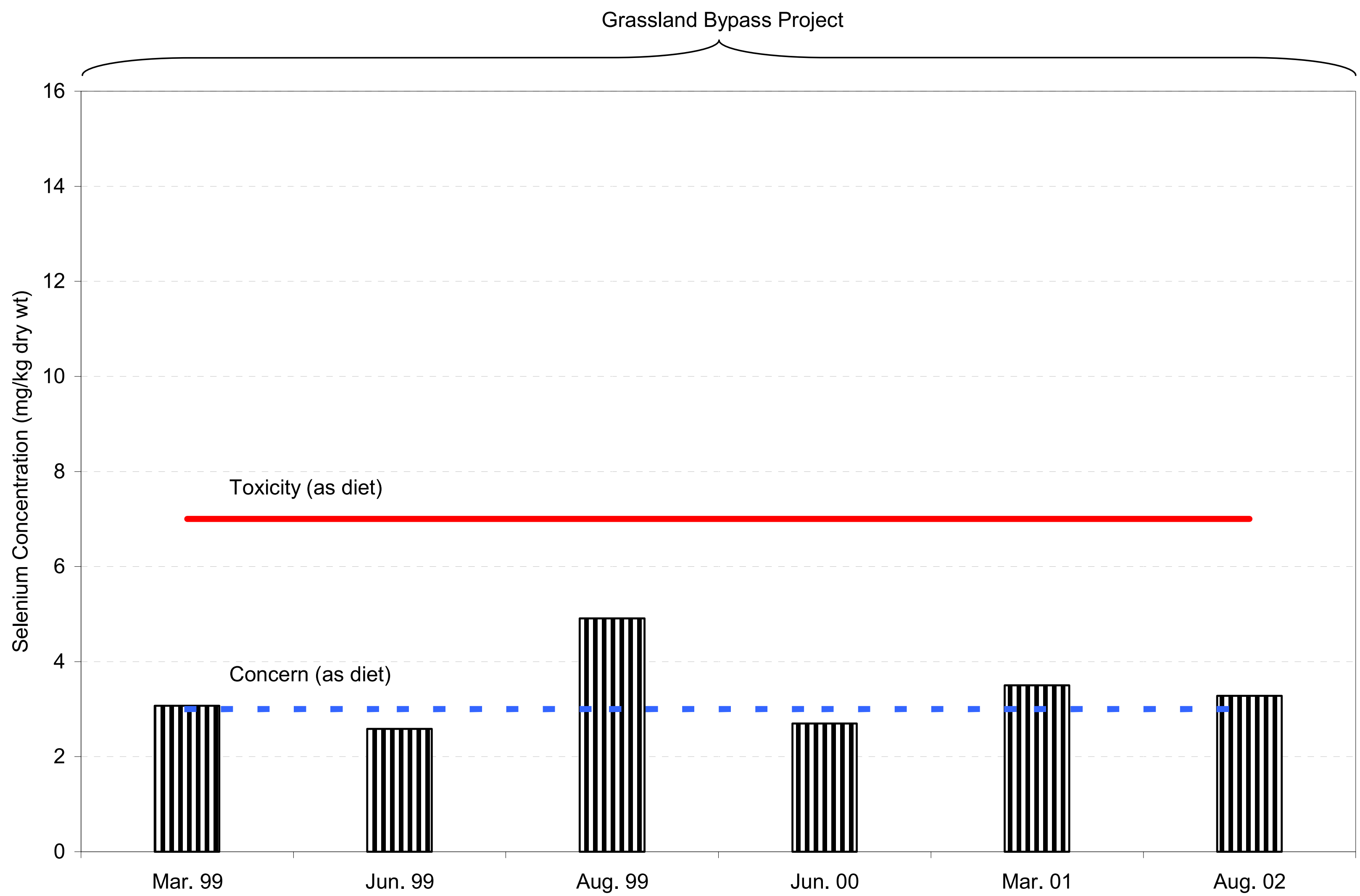


Figure 9. Selenium in invertebrates in Mud Slough above the San Luis Drain discharge (Site C).

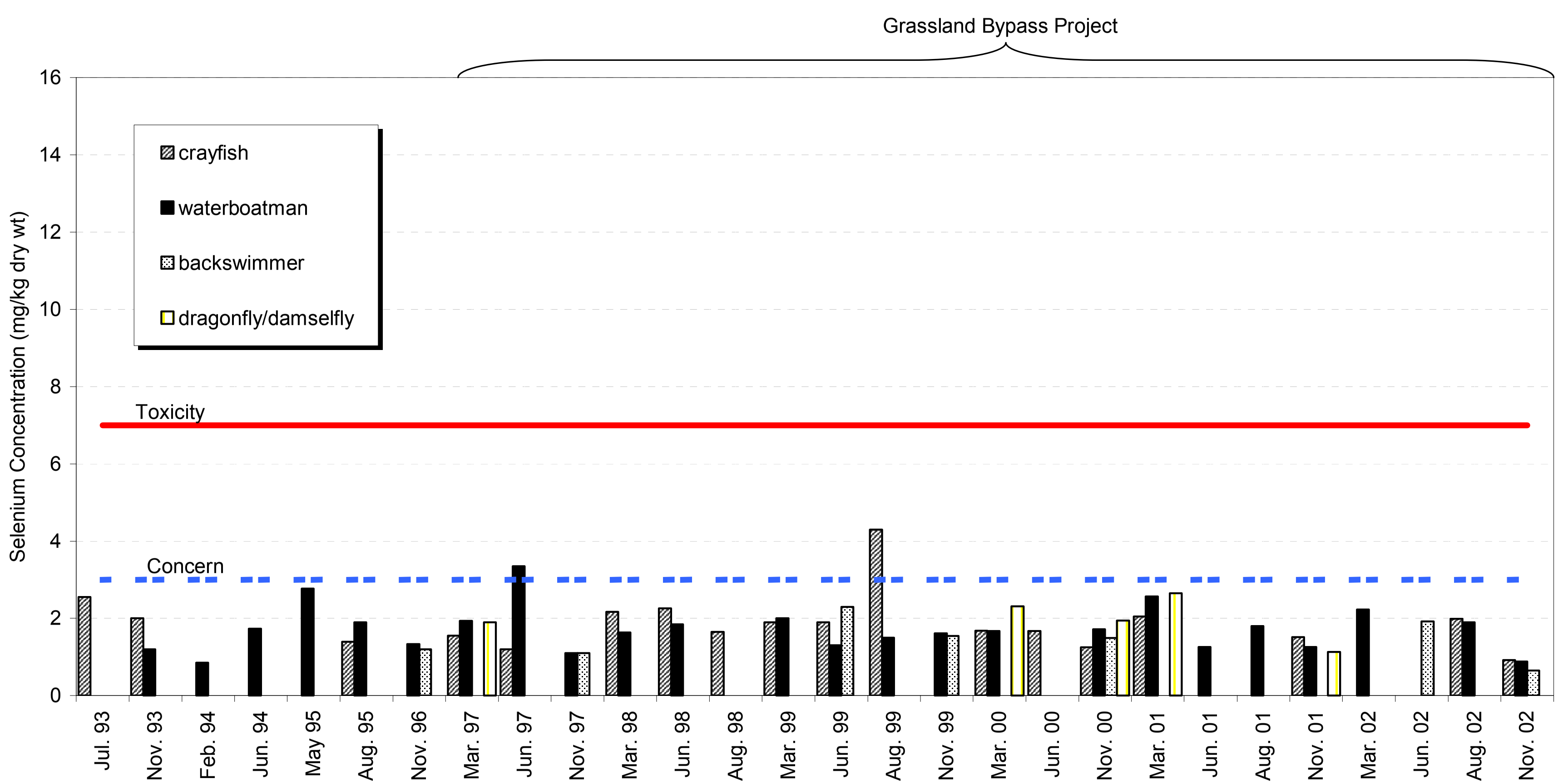


Figure 10. Selenium in small fish in Mud Slough below the San Luis Drain discharge (Site D).

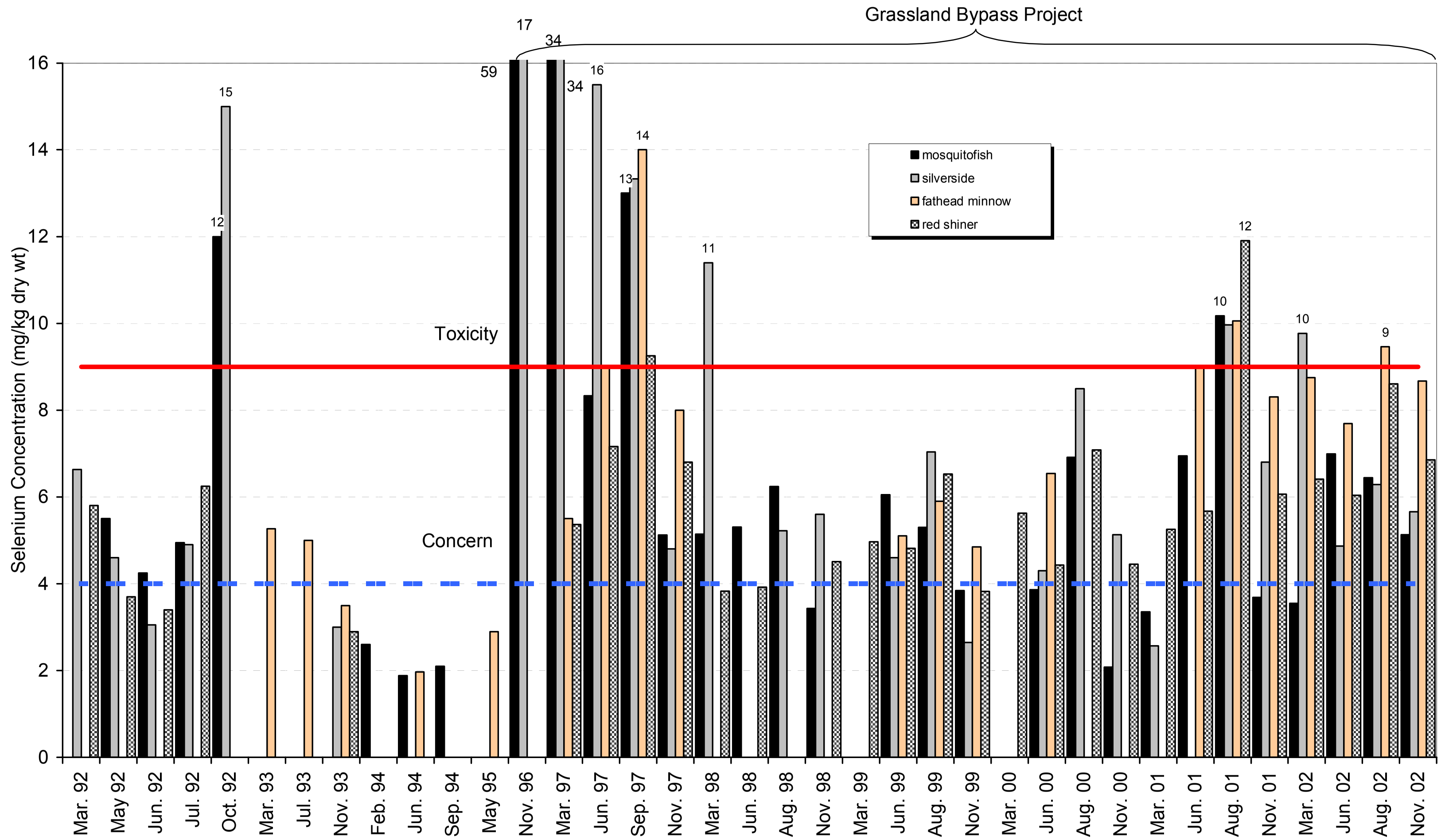


Figure 11. Selenium in medium-size fish in Mud Slough below the San Luis Drain discharge (Site D).

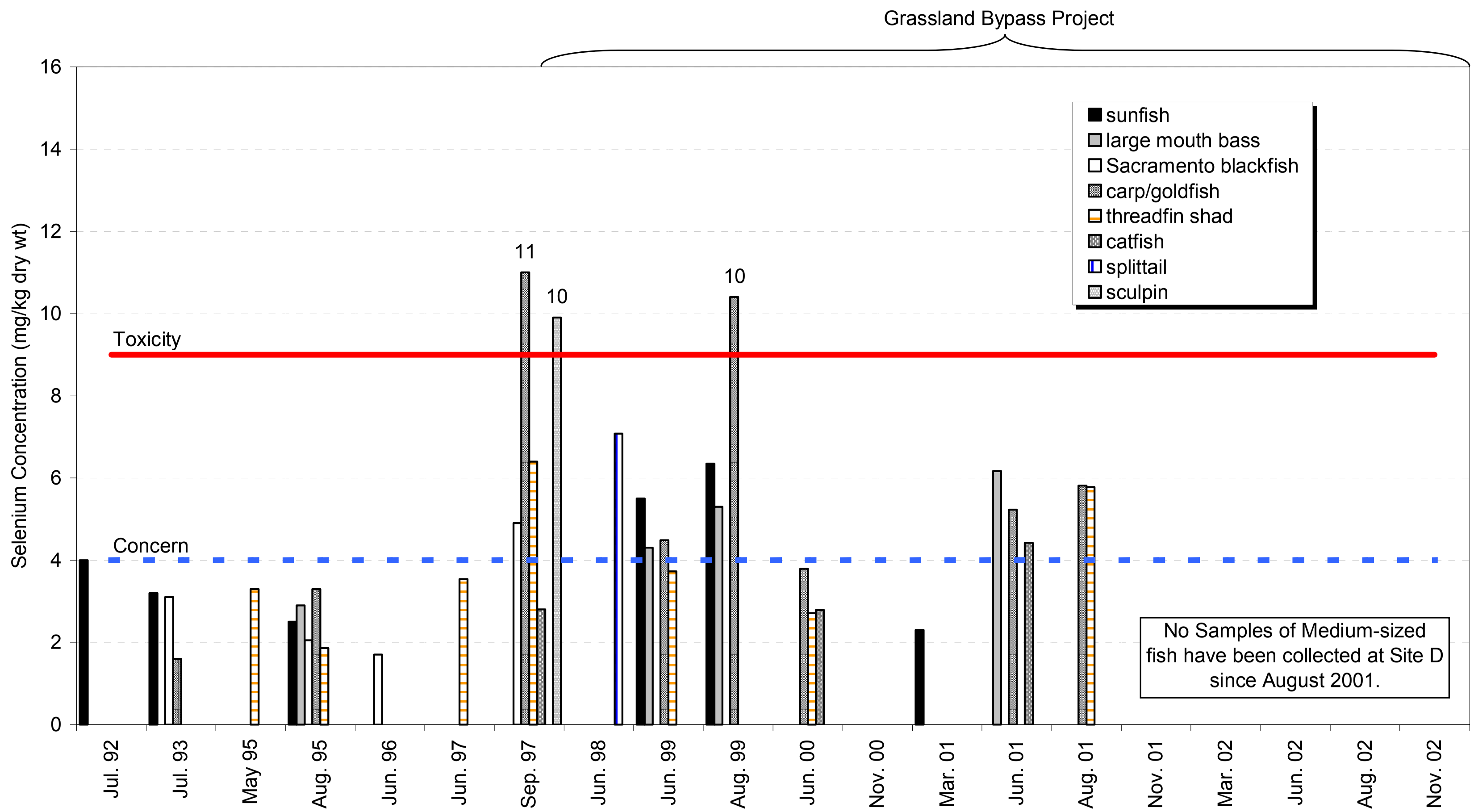


Figure 12. Selenium in bullfrog tadpoles in Mud Slough below the San Luis Drain discharge (Site D).

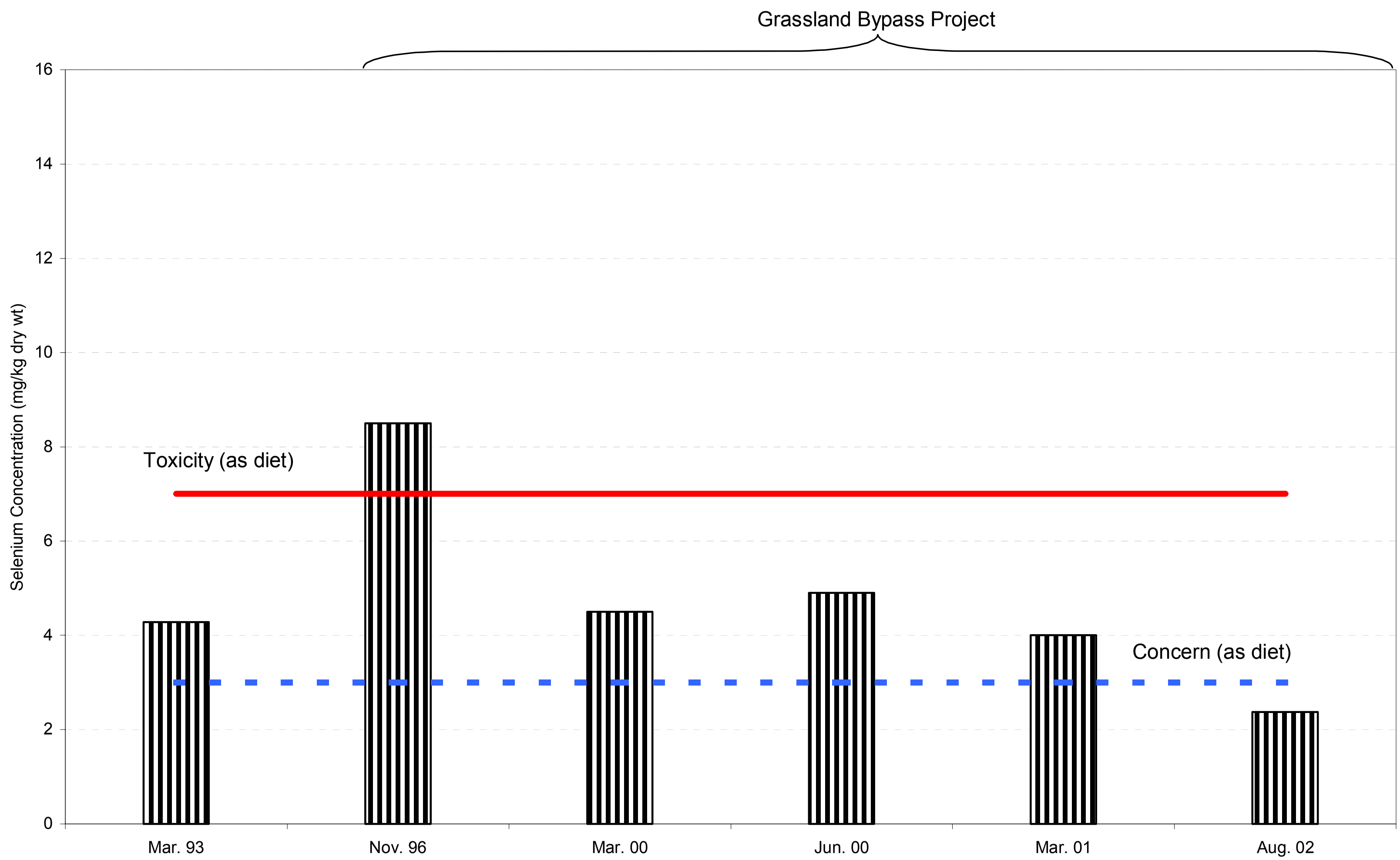


Figure 13. Selenium in invertebrates in Mud Slough below the San Luis Drain discharge (Site D).

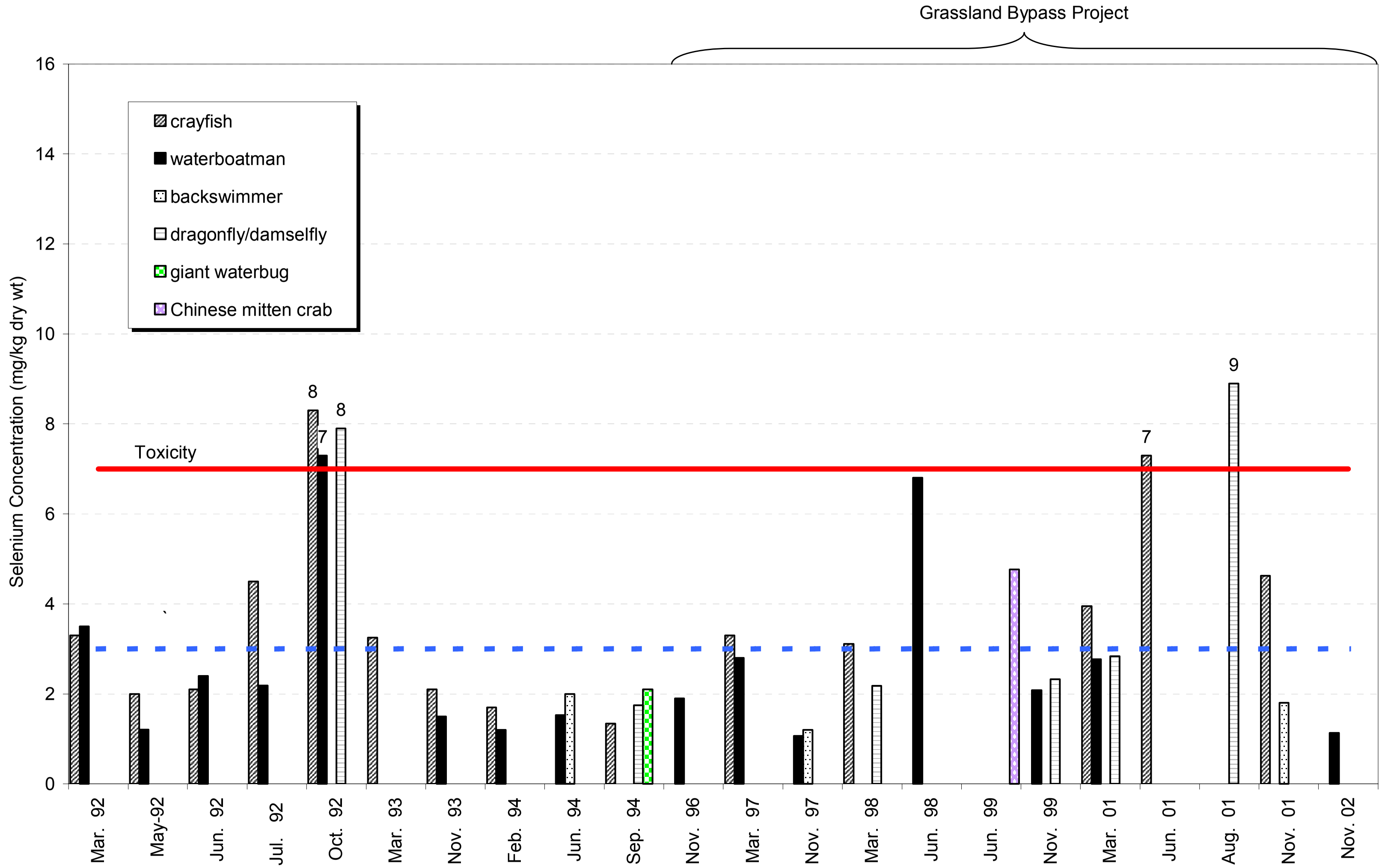


Figure 14. Selenium in small fish in a Mud Slough backwater below the Drain discharge (Sites I and I2).

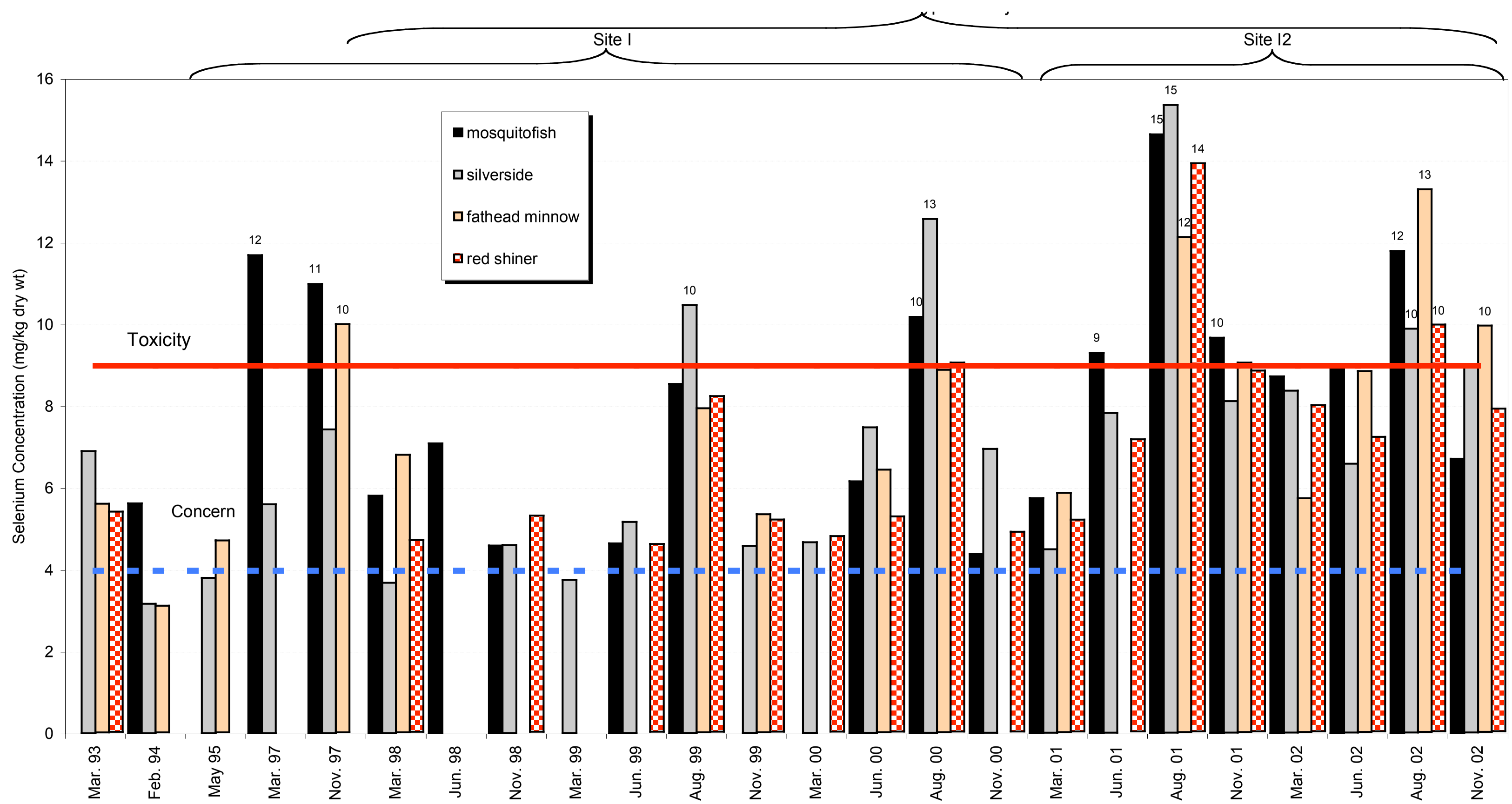


Figure 15. Selenium in medium-size fish in a Mud Slough backwater below the Drain discharge (Sites I and I2).

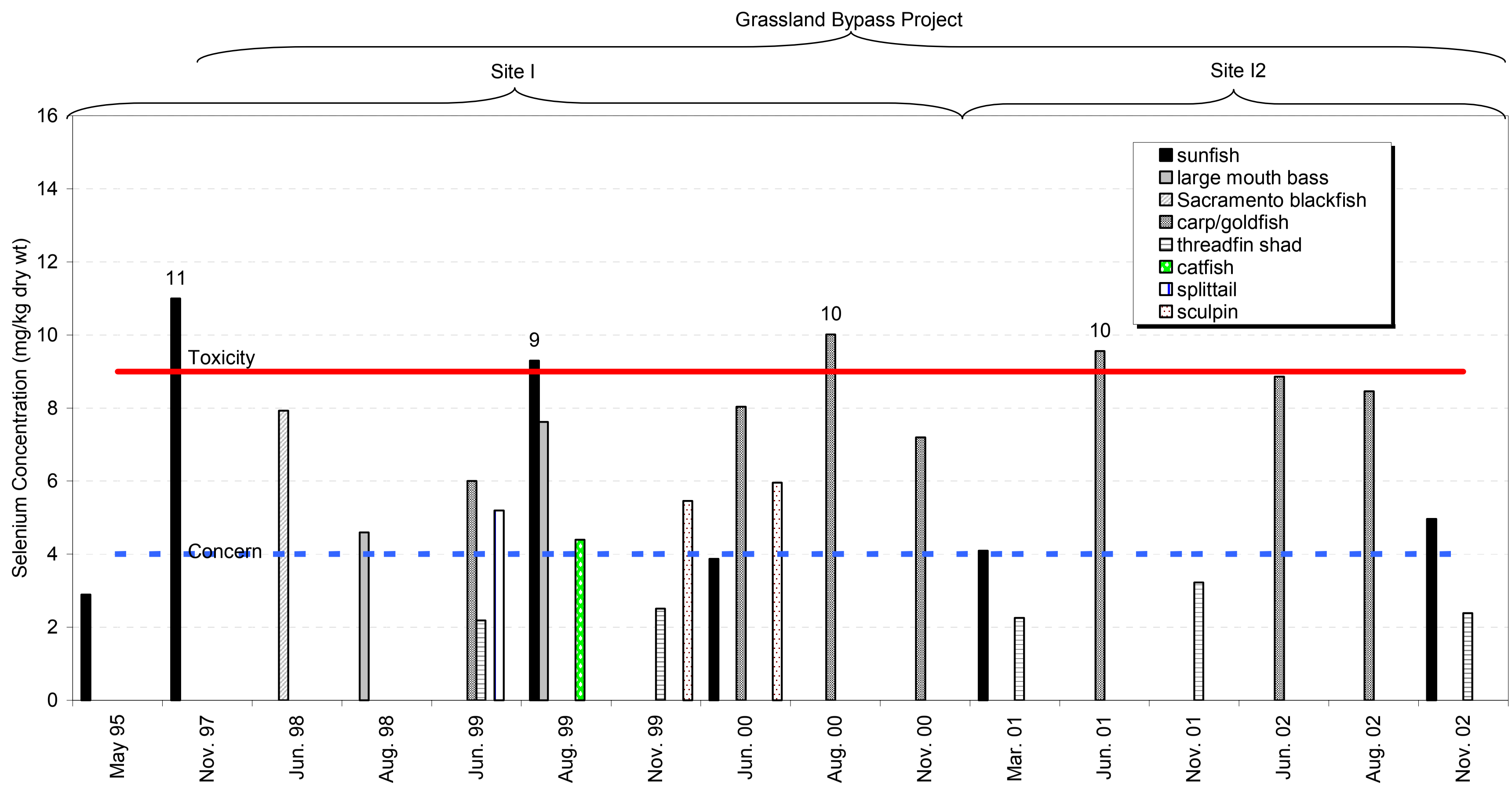


Figure 16. Selenium in invertebrates in a Mud Slough backwater below the Drain discharge (Sites I and I2).

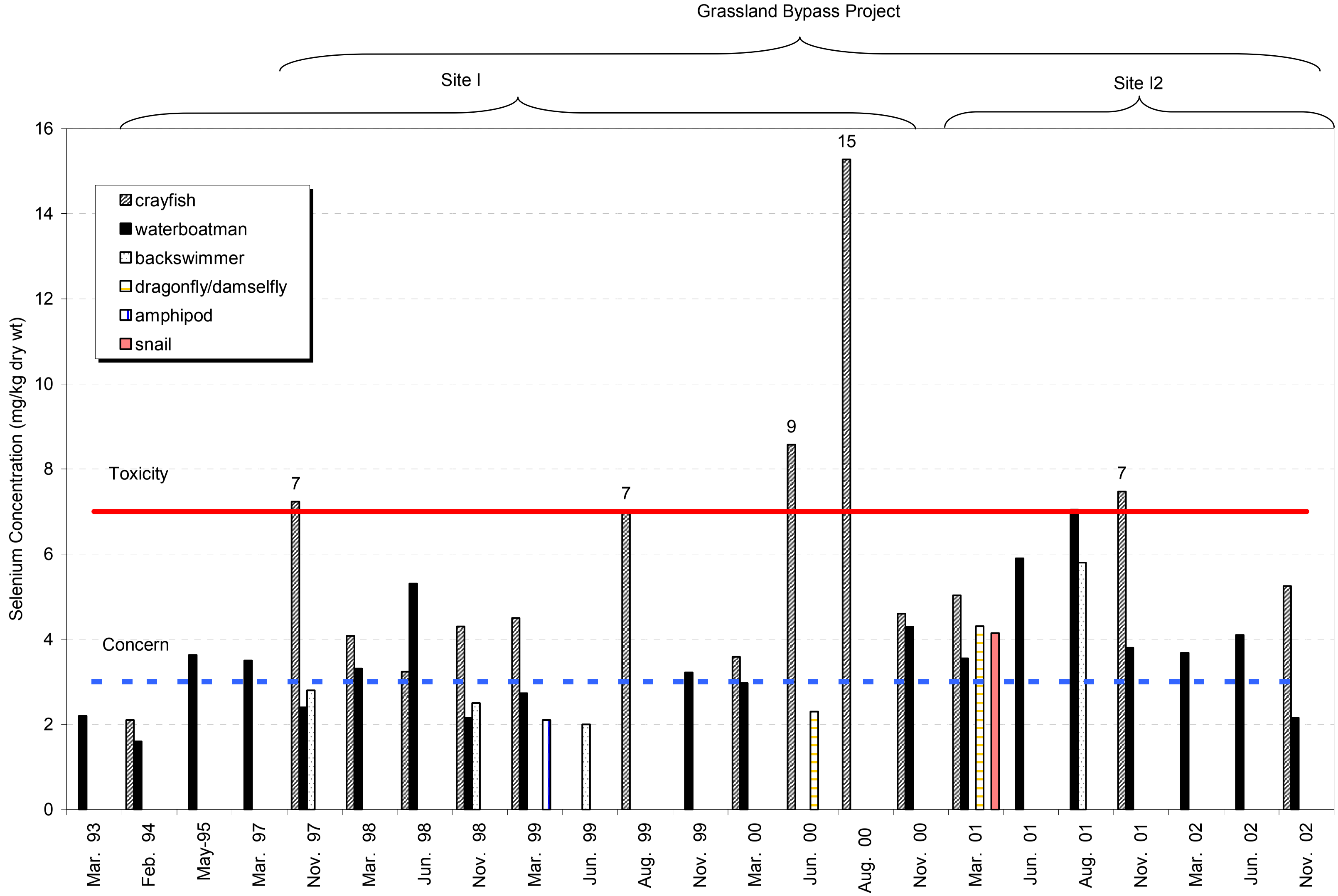
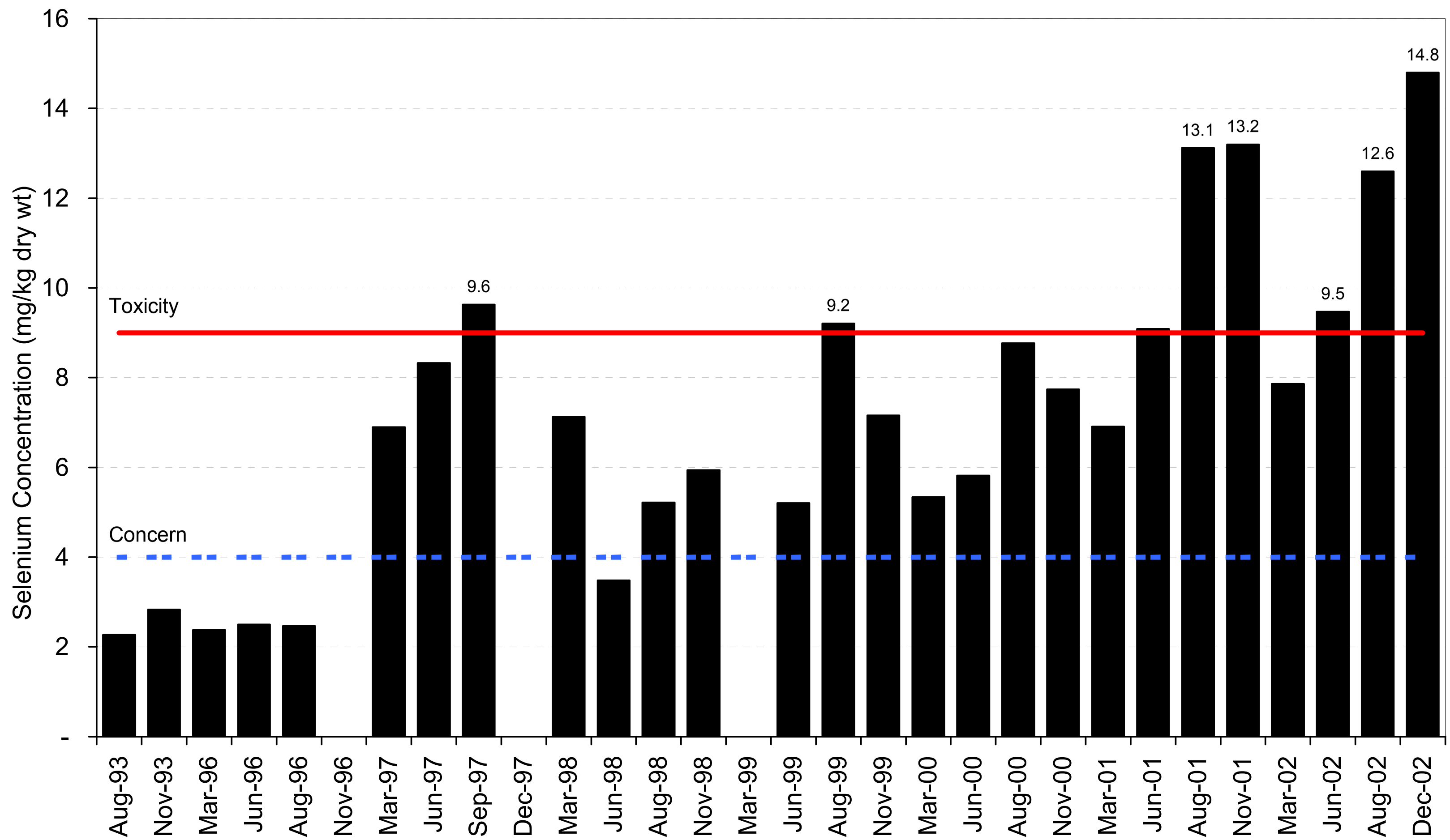


Figure 17. Selenium Concentrations in Whole-Body Fish* Tissue from Mud Slough at Hwy 140 (Site E).



* Mosquitofish *Gambusia affinis* See previous reports for other species

Figure 18. Selenium Concentration in Invertebrates from Mud Slough at Hwy 140 (Site E).

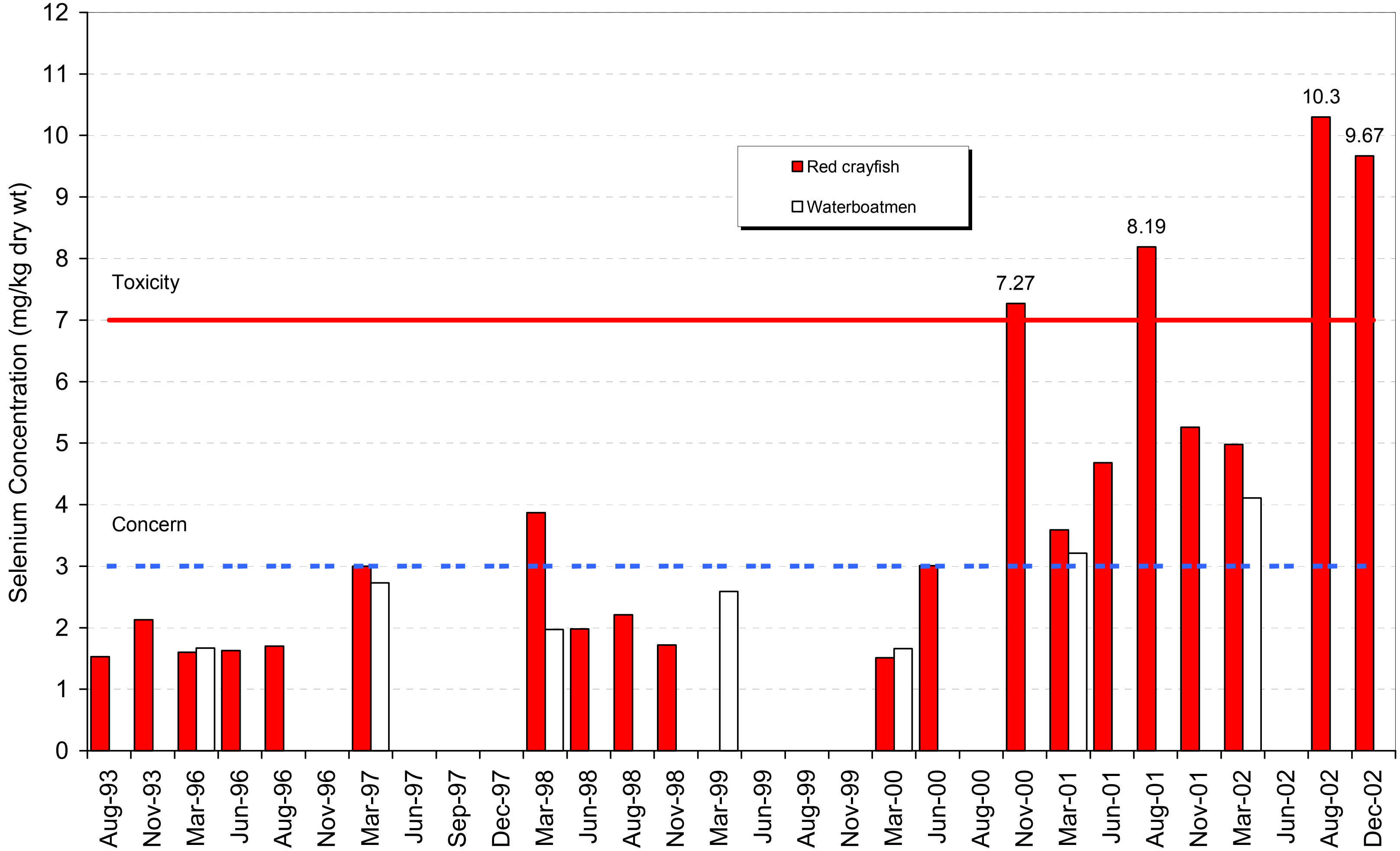
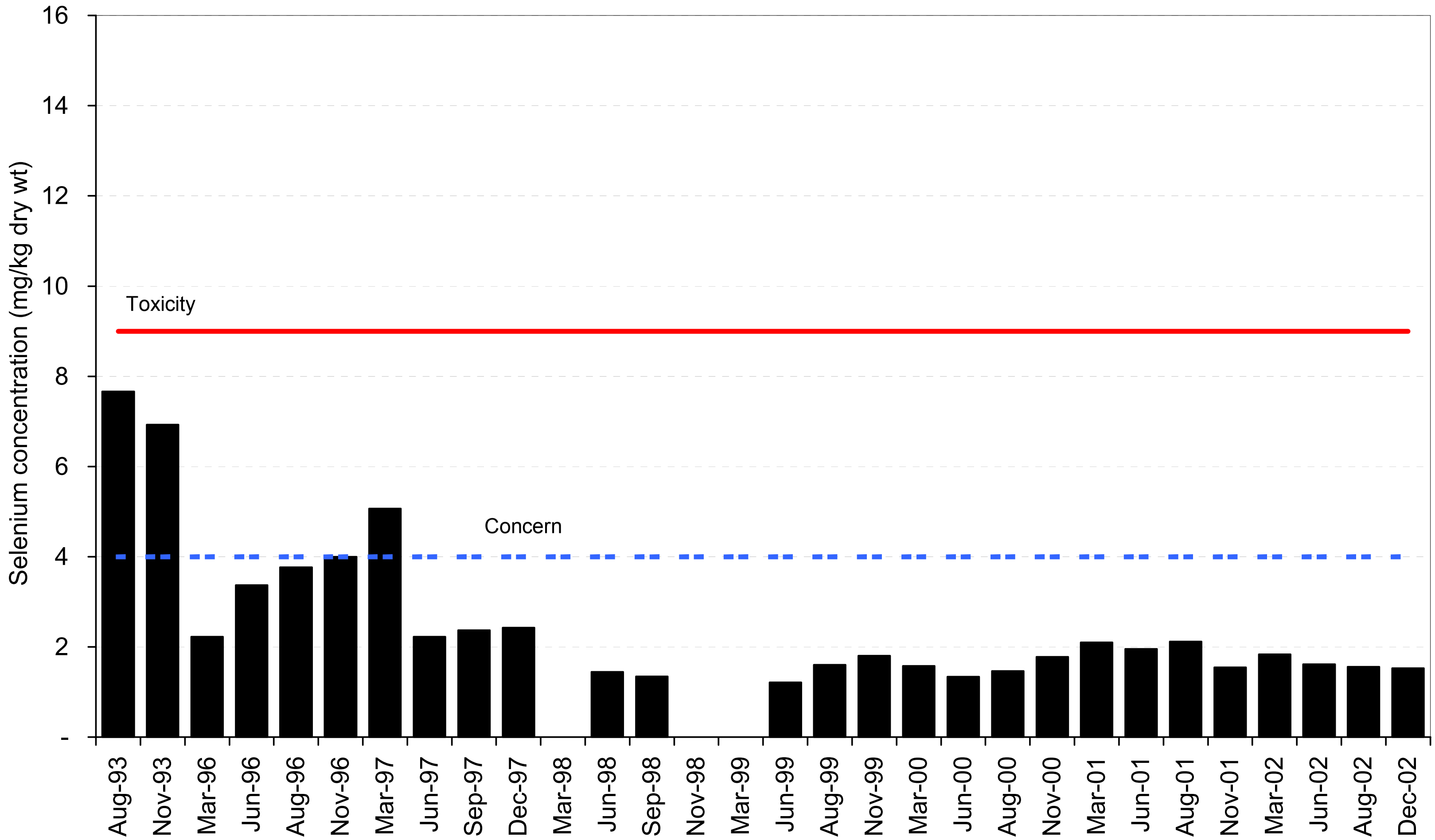


Figure 19. Selenium Concentrations in Whole-Body Fish* Tissue from the San Joaquin River Upstream of the Mud Slough Confluence (Site G).



* Mosquitofish *Gambusia affinis* See previous reports for other species

Figure 20. Selenium Concentration in Invertebrates from the San Joaquin River Upstream of the Mud Slough Confluence (Site G).

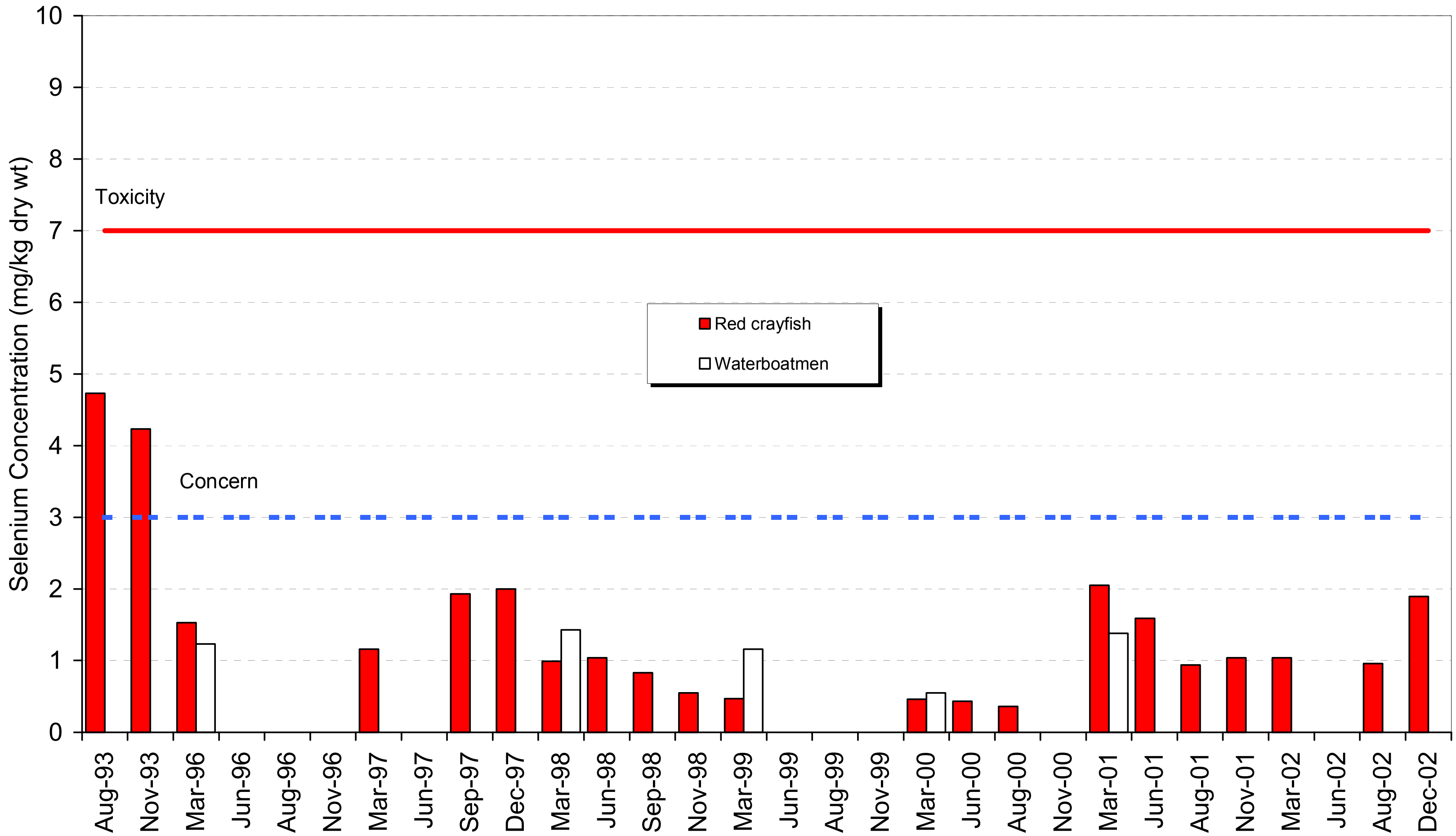
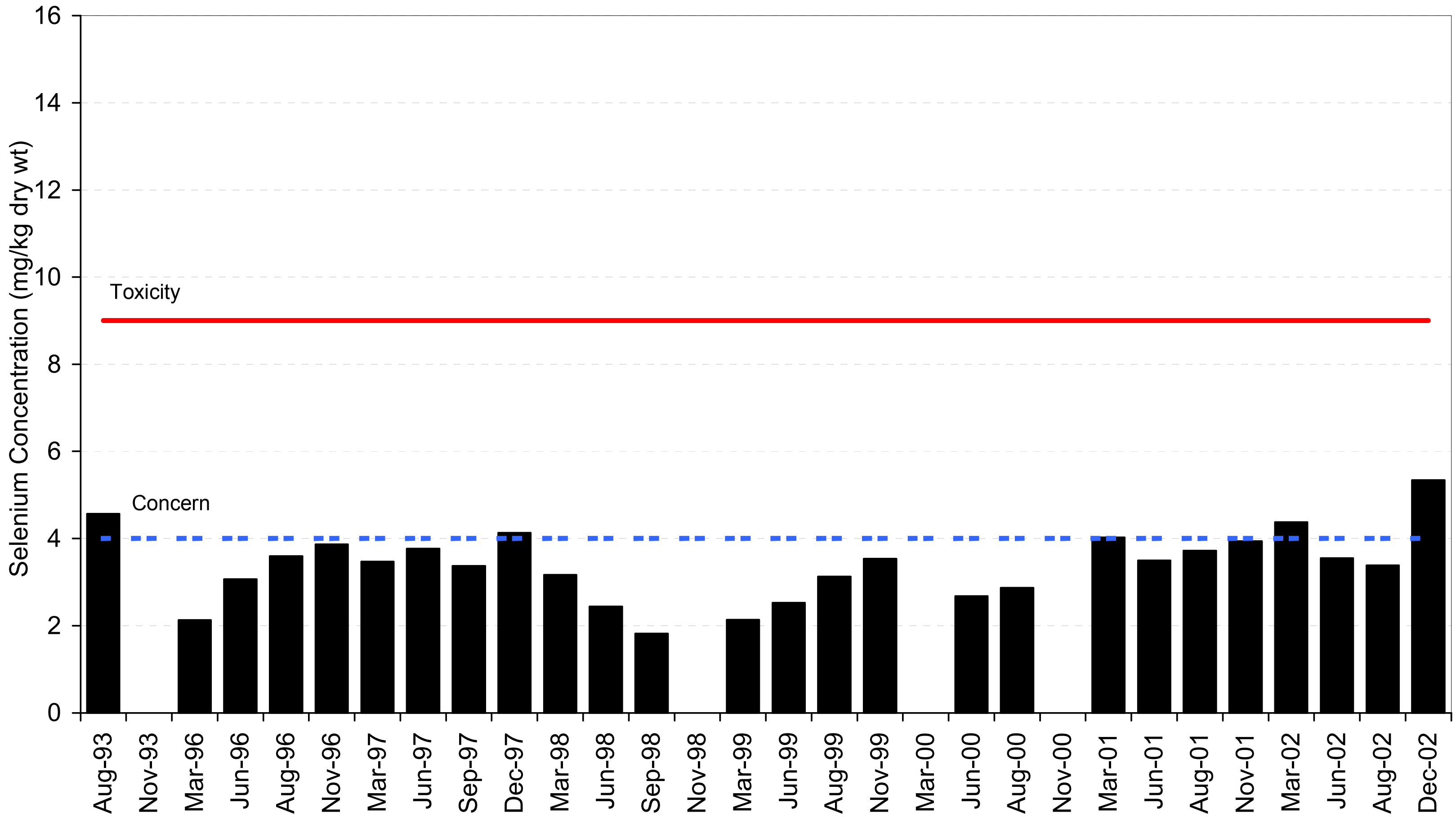


Figure 21. Selenium Concentrations in Whole-Body Fish* Tissue from the San Joaquin River Downstream of the Mud Slough Confluence (Site H).



* Mosquitofish *Gambusia affinis* See previous reports for other species

Figure 22. Selenium Concentration in Invertebrates from the San Joaquin River Downstream of the Mud Slough Confluence (Site H).

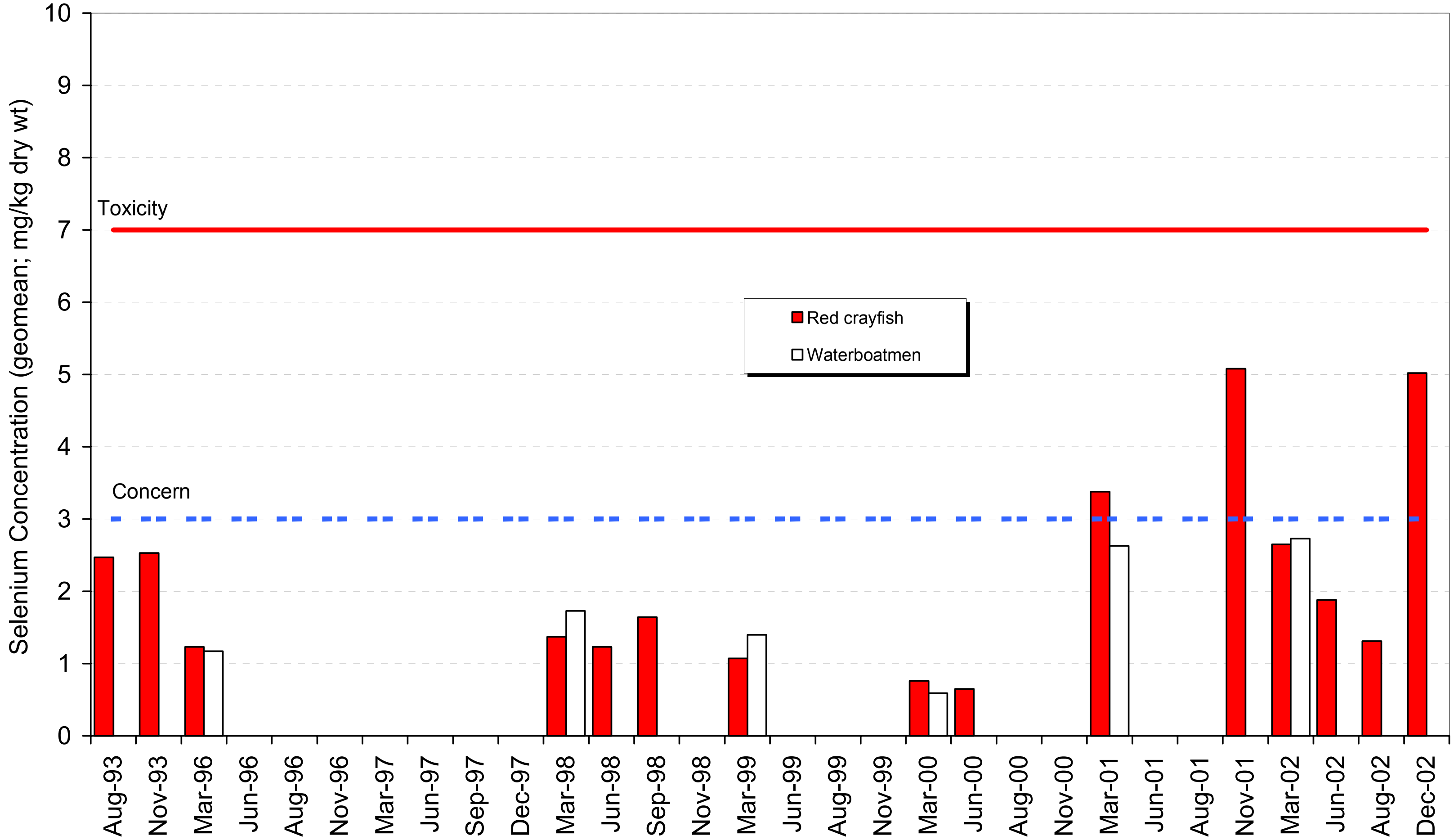


Figure 23. Percent abundance of trophic classifications over time at Site E
August 1993 - December 2002

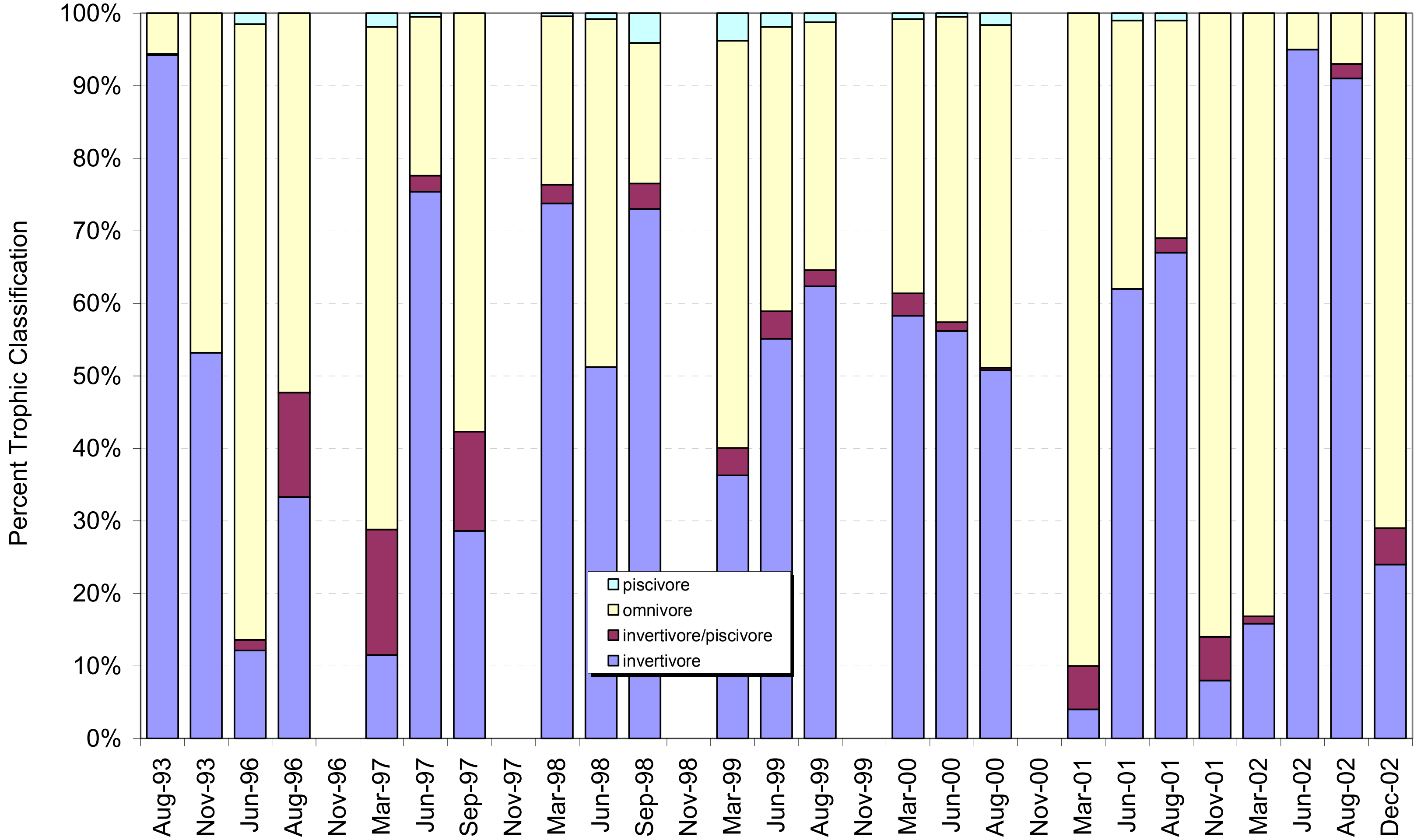


Figure 24. Percent abundance of trophic classifications over time at Site G August 1993 - December 2002

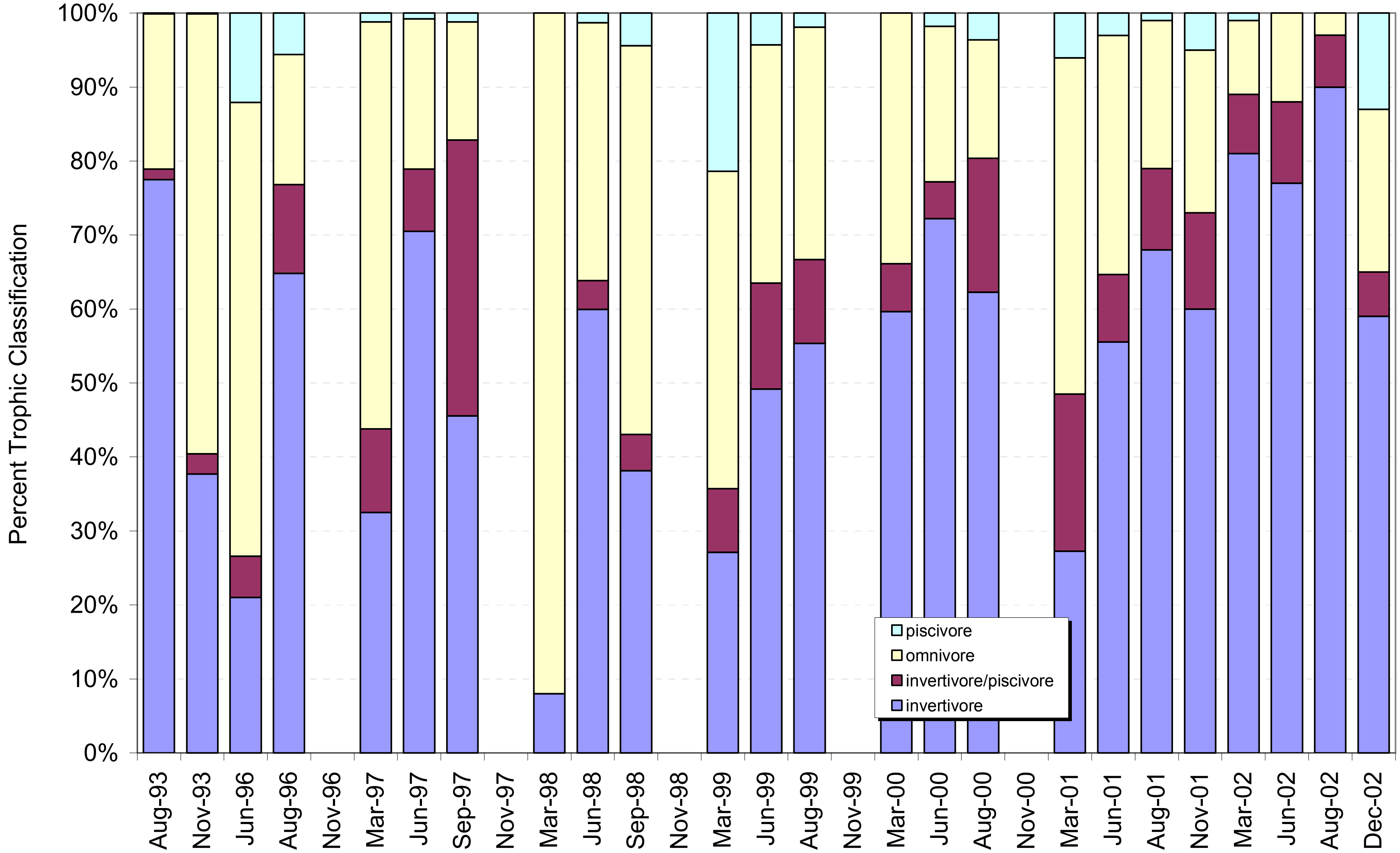


Figure 25. Percent abundance of trophic classifications over time at Site H
August 1993 - December 2002

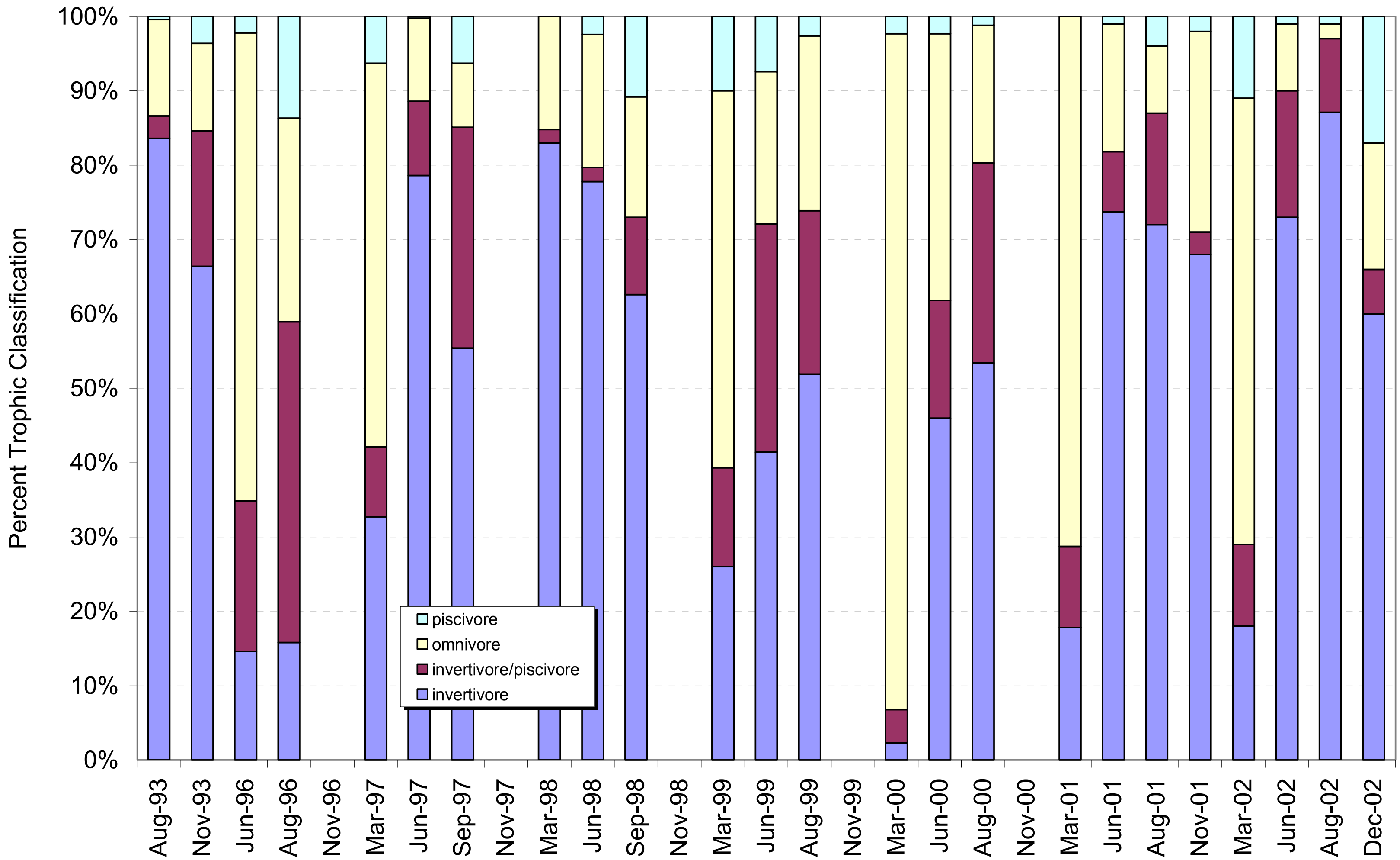


Figure 26. Observed Anomalies* in all Fish Species Caught at Sites E, G, and H**

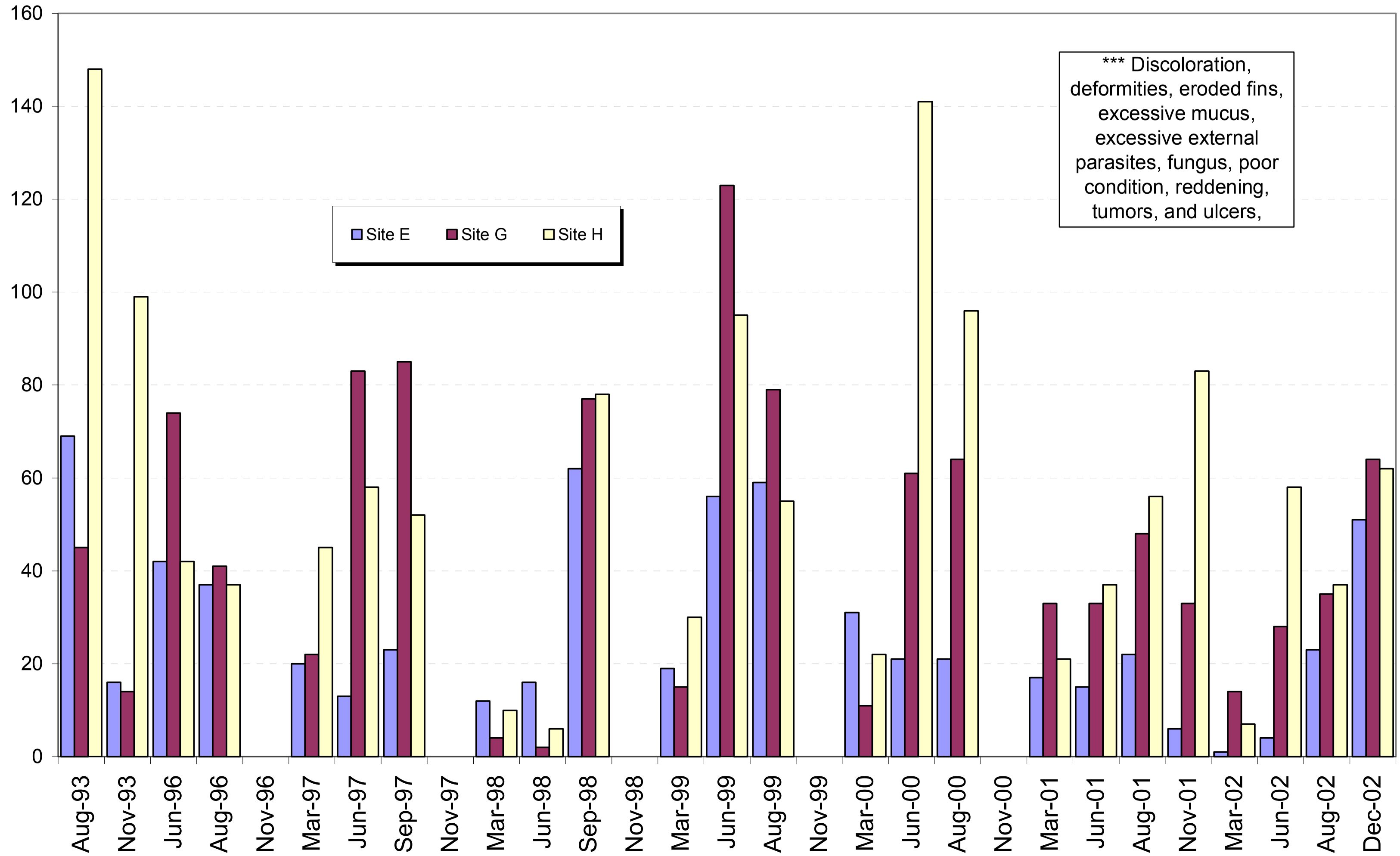
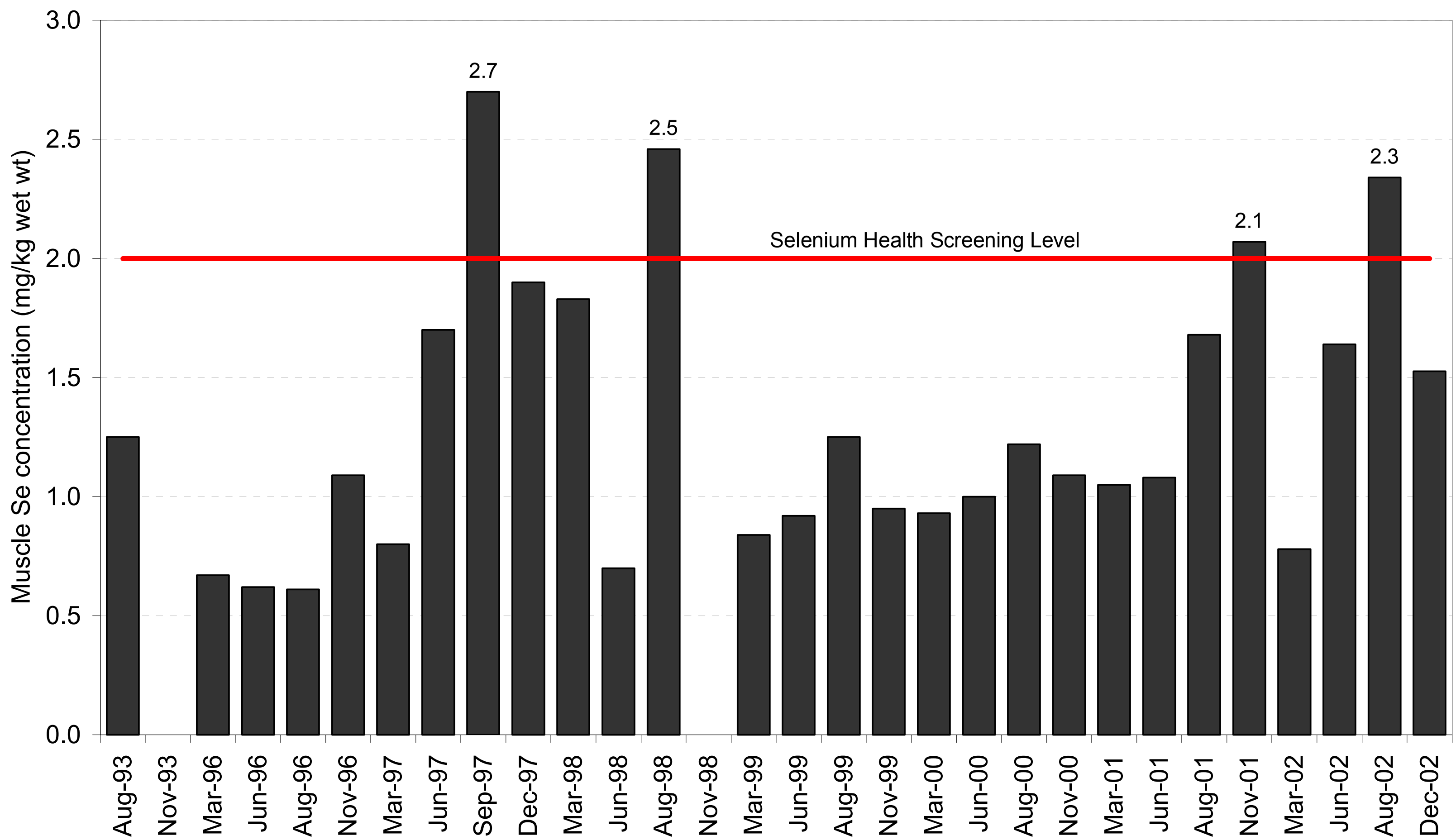
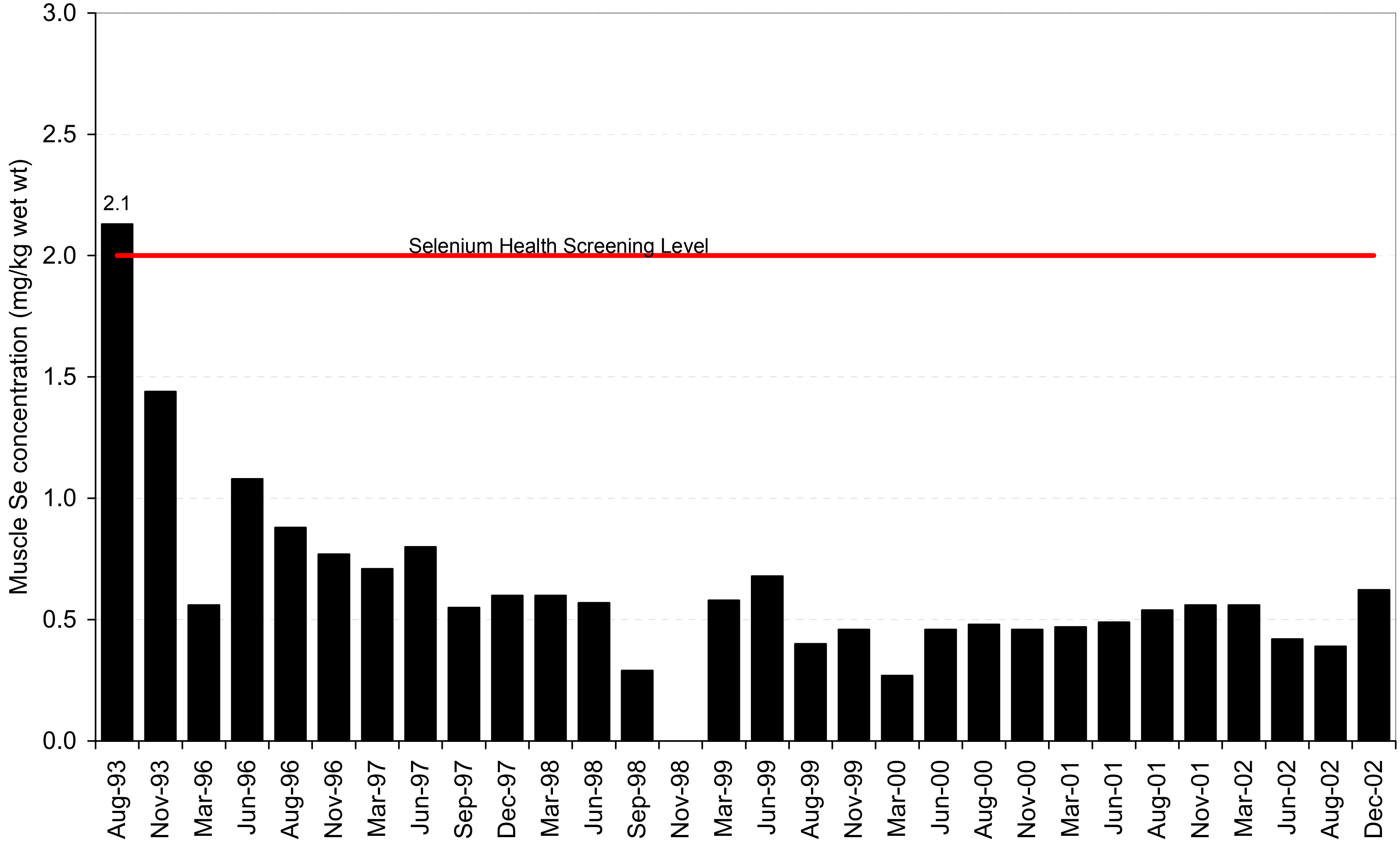


Figure 27. Selenium Concentrations in Fish Muscle Tissue from Mud Slough at Hwy 140 (Site E).**



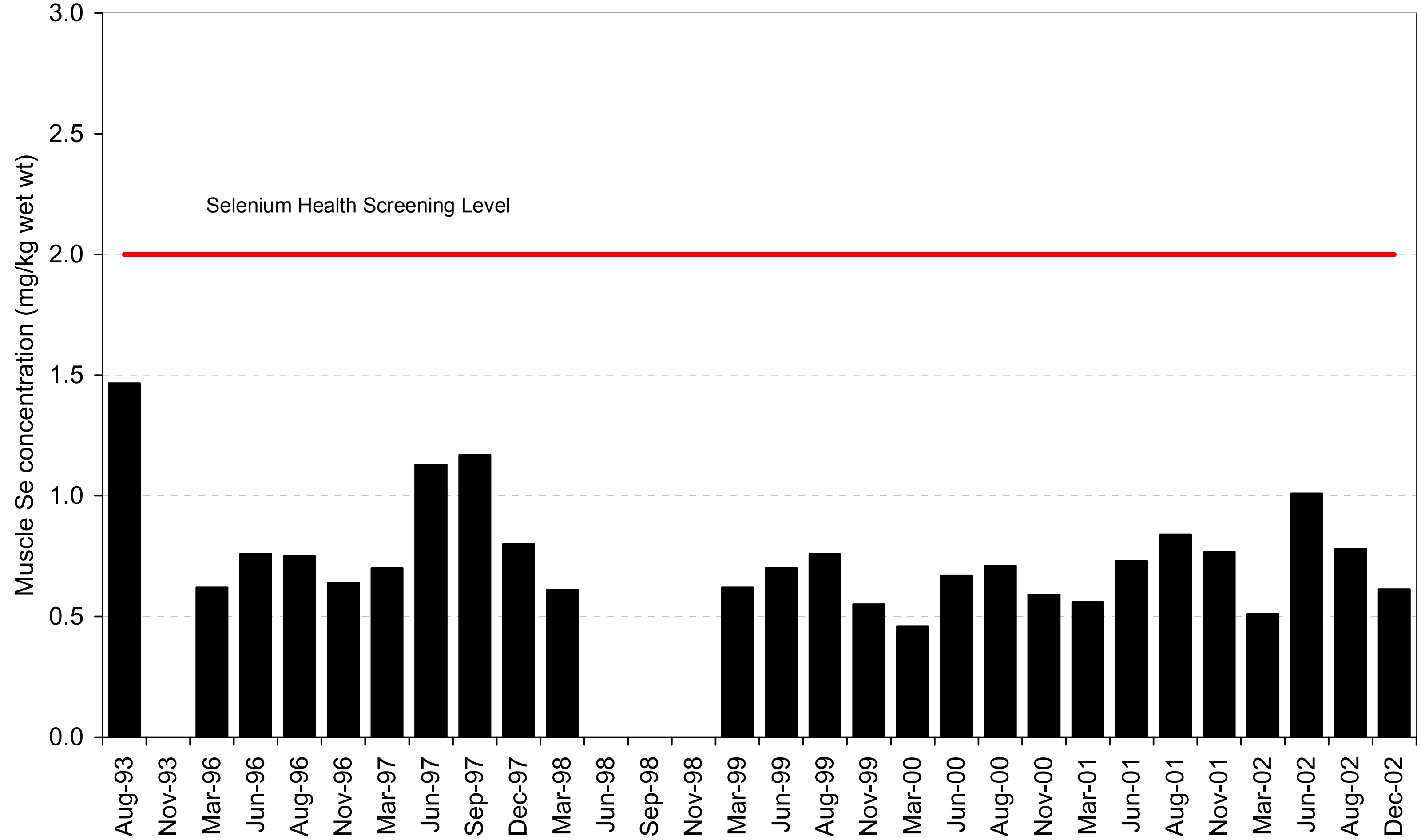
** Carp *Cyrpinus carpus* {See previous reports for other species}

Figure 28. Selenium Concentrations in Fish Muscle Tissue from the San Joaquin River Upstream of the Mud Slough Confluence (Site G).**



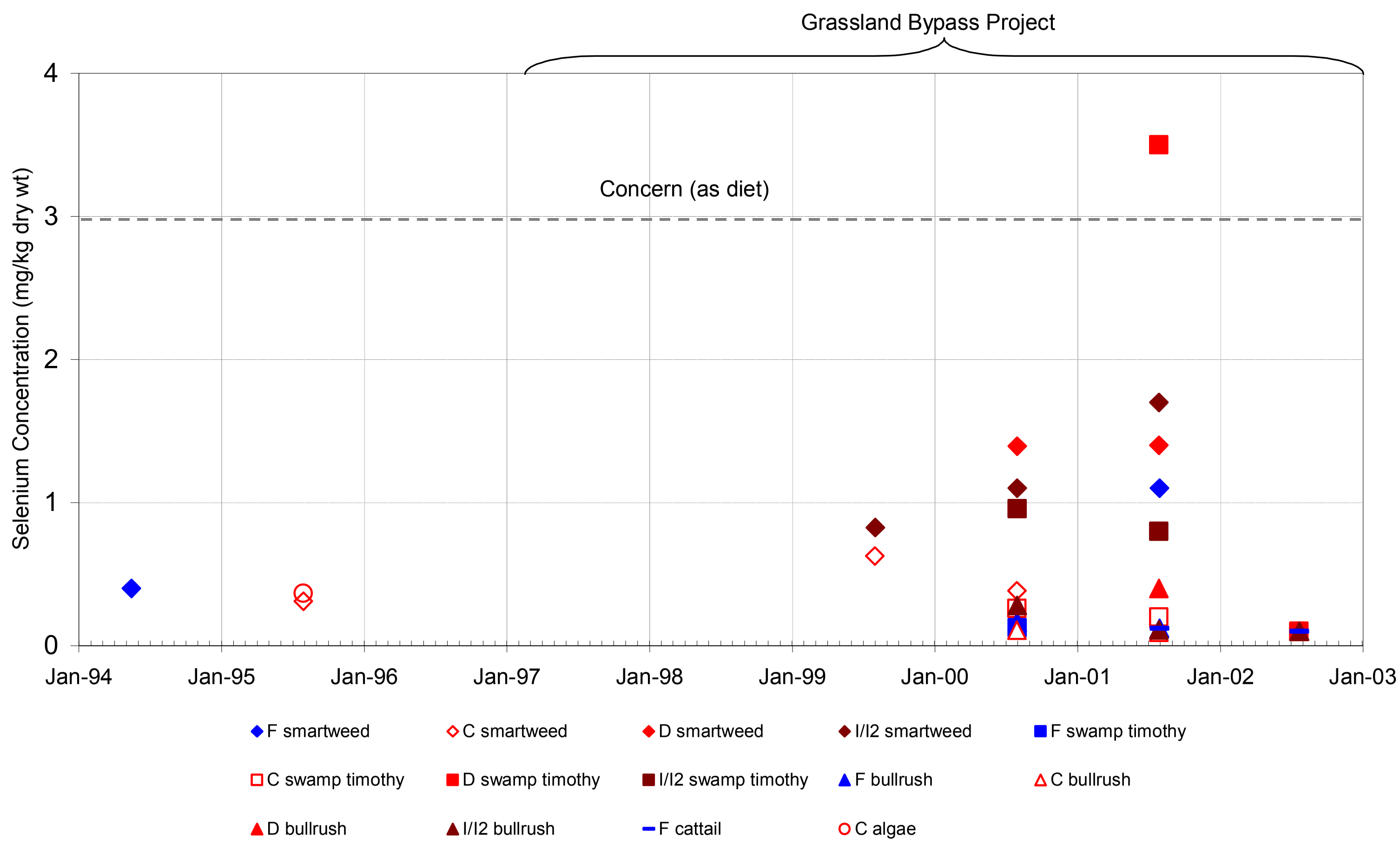
** Carp *Cyrpinus carpus* {See previous reports for other species}

Figure 29. Selenium Concentrations in Fish Muscle Tissue from the San Joaquin River Downstream of the Mud Slough Confluence (Site H).**



** Carp *Cyrpinus carpus* {See previous reports for other species}

Figure 30. Selenium in plants (seed heads where applicable)



All samples of vegetation collected in August of 2002 at Sites F, C, D and I2 were below the reporting limit (0.20 mg/kg, dry wt.) for selenium.

Figure 31. Selenium in bird eggs